IMPERIAL BUREAU OF MYCOLOGICAL INSTITUTE
28 MAY 1940

THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY

Vol. 22, N.S., Nos. 18–24. MAY, 1940.

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[*Authors alone are responsible for all opinions expressed in their Communications.*]

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,

BALLSBRIDGE, DUBLIN.

HODGES, FIGGIS, AND CO.,

20 NASSAU STREET, DUBLIN.

WILLIAMS AND NORGATE,

36 GREAT RUSSELL STREET, LONDON, W.C. 1.

1940.

Price Six Shillings.

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No. 18.

ON A RECENT BOG-FLOW IN POWERSCOURT MOUNTAIN TOWNLAND, CO. WICKLOW.

By A. D. DELAP and G. F. MITCHELL.

[Read June 20. Published separately December 19, 1939.]

The flow which is described in this paper was reported to one of the authors by Miss A. V. Delap, who saw it first on July 25th, 1938. In the investigation and survey that followed the discovery Messrs. A. Farrington, E. C. Allberry, and B. J. Yarr assisted. For this co-operation the authors wish to express their thanks.

The Military Road from Glencree to Sally Gap crosses the ridge that connects Kippure (754 m.) with Tonduff (640 m.). To do this it first hugs the side of the main valley, passing just below the eastern margin of Lr. Lough Bray, and then strikes up the valley of a small tributary stream flowing from the south. It follows this valley to the source of the stream at an altitude of 510 m. From thence the summit of the ridge (523 m.) is easily reached. The stream receives from the west the outflow from Upr. Lough Bray.

Above 460 m. the stream flows in a narrow trench that it has cut into the decomposing surface layers of the granite of which the ridge is composed. Above this altitude the gentle slopes of the granite are covered by a mantle of peat about 215 cm. in thickness. The peat is firm underfoot, and the surface vegetation is that which is typical of such mountain bogs, *Scirpus*, *Eriophorum*, and *Calluna* being prominent. Banks of peat overhang both sides of the course of the stream.

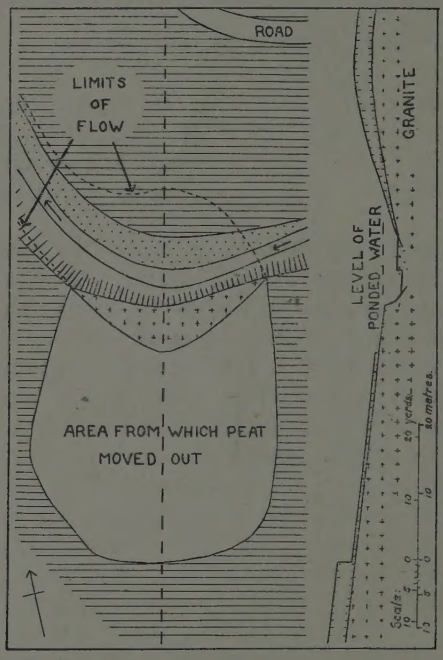
The flow took place on the western side of the stream about 200 m. below its source, at an altitude of *ca.* 490 m., where the peat sloped down towards the stream at an angle of *ca.* 6°. Here over a frontage of about 35 m. the peat bank had slumped forwards, and had been followed by the bulk of the peat out of an area of the same width and a depth of 40 m. Some large sods which had moved forwards a short distance were stranded in the area, to which incompletely detached masses formed a jagged rim. The excavated area, except for the portion immediately above the stream, was floored by firm greasy peat, through which bosses of granite protruded here and there. The surface of the floor was marked by a series of parallel grooves, which had been engraved by the forward movement of the upper layers. Immediately above the stream all the peat had been swept away, and the surface of the decomposed granite exposed.

Examination of the peat profile in the upper margin of flow revealed the following stratification:—

0– 80 cm. ... Fresh brown *Sphagnum-Eriophorum* peat, with remains of Calluna.

195–215 cm. ... Well humified brown cheesy peat, with little plant structure preserved.

215 + cm. ... Granite sand.



As the peat was firm to its base, there had been no escape of water from the lower layers, nor had these flowed out from underneath the upper layers above the actual area of the flow. Along the upper margin of the flow water was trickling out of the peat face about the level where a change in texture took place, *ca.* 15 cm. above the base. This line of seepage may indicate the plane along which movement took place, for, as described above, the basal layer of the peat remained *in situ* over the greater part of the excavated area. Large cracks were to be seen round the upper margin, but they did not extend to any great distance from it, nor had they any regular arrangement. There was no welling-up of softer peat between the cracks.

When the solid peat slipped forwards and downwards its progress was arrested by the rising ground at the far side of the stream course, and it came

to rest across the bed of the stream. It must have formed a temporary dam, for there was evidence of the ponding up of water above the site of the flow to a height of 238 cm. above the normal level.

The features presented by the stream course below the flow are quite consistent with having been caused by the sudden spate of water released by the carrying-away of the temporary peat dam. The transported rafts of peat merely aggravated the effects of the velocity, and the fragments which were shed as the debris was carried along enable the limits of the flood to be fixed with some accuracy.

In the narrow channel as far as the 460 m. level the flood rose to a height of about 4 m., and the bed of the stream was scoured and stripped of all loose debris. Below this level the stream descends abruptly for a distance of about 12 m. by a series of cascades, and then traverses a more level open area. As the flood swept down the cascades scouring of the valley sides and floor was especially vigorous, and as the valley opened out the flood had again mounted the sides to a height of about 4 m. When the flood spread out in the flatter area erosion became less vigorous, and blocks of granite torn from the channel higher up were deposited together with rolled sods of peat up to 60 cm. in diameter. Here the flood had a breadth of about 50 m. The water had surged up the outlet from Upr. Lough Bray for a short distance, and had left a convex arc of debris to mark its furthest advance. This more level area was covered by a growth of rushes and bracken, and the stems of these plants must have retarded the flow effectively. For, when the stream passes under the road in a culvert at about 400 m., there had been no lateral flooding, though there was evidence of a rise of about 90 cm. in the stream level. A short distance above the culvert the last rolled sods, about 30 cm. in diameter, were seen. Below the culvert the flood had been confined to the channel of the stream.

DISCUSSION.

The peat which clothes the hill slopes in this area must be continually tending to slide towards the stream bed under the influence of gravity. This tendency is resisted by the firm banks of dried peat which overhang the sides of the channel. Failure of the peat bank will allow the peat to slide. This failure might be caused either by the thrust due to gravity overcoming the resistance of the bank, or by the stream undercutting the bank. Heavy rainfall in the first week of July, 1938 (45 mm. were recorded in Dublin city), may have brought both these factors into play. The thrust of the peat was increased by the weight of the water retained on its upper surface, and the volume of the river was augmented by the rain. It is worth noting that the flow took place on the outside of a bend in the stream where undercutting might be expected to be most vigorous.

The collapse of the bank allowed the solid peat to slump forward and block the course of the stream. At this stage the bog-flow proper comes to an

end, for the transport and distribution of its debris were due to the rush of the pent-up water when set free by the yielding of the peat dam. The volume of water involved has been estimated at 850 cub. m. Though this quantity of water is relatively trivial, and the valley immediately below uninhabited, such a peat dam on a larger scale might cause serious damage. One is reminded of avalanche-dammed streams in Switzerland, and the vigilance of the authorities there to have such dams removed before the impounded water has reached any large volume.

In being caused by the yielding of a bank of peat this flow resembles the one that took place in Co. Clare in 1934 (1). In that instance, however, the peat bank occurred above a rock escarpment, and there was no question of undercutting of the bank being involved. The nature and stratification of the peat concerned in this flow are very similar to that of the flow in Co. Wicklow in 1937 (2). In that case also firm peat, with a considerable amount of plant fibre preserved, had slipped forward partly over, and partly lubricated by, amorphous greasy peat at the base. Further, in this flow no great volumes of water were released by the rupture of the upper layers of the bog. Water was involved in the flow, but this was provided by the stream into whose course the flow took place, and not by the flow itself.

From investigations of recent bog flows it is becoming increasingly apparent that the mantles of peat that blanket the mountain slopes are very sensitive to the influence of gravity. Where the peat is firm to its base actual slipping can take place on a slope which need not exceed 6°, while when the under layers are semi-fluid a burst, followed by violent flow, can occur on a slope of 4° (1, p. 250; 3, p. 183).

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2. Mitchell.—Scient. Proc. R.D.S., **22**; No. 4, 1938.
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No. 19.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND.

LECANORA GANGALEOIDES—PART 2.

BY T. J. NOLAN, B.A., D.SC.,
AND
J. KEANE, PH.D.,
University College, Dublin.

[Read JANUARY 23. Published separately FEBRUARY 20, 1940.]

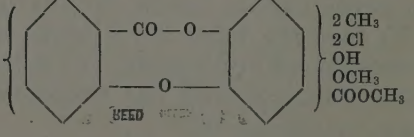
INTRODUCTION.

IN Part 1 of this investigation (Sci. Proc. R.D.S., **21**, p. 141) an account was given of some preliminary results obtained in our examination of the lichen *Lecanora gangaleoides*. It was stated that the principal constituent of the lichen is a chlorine-containing depsidone which we call gangaleoidin and to which the formula $C_{18}H_{14}O_7Cl_2$ is given; that atranorin and chloratranorin were also present; that there appeared to be evidence of the presence of a substance with a higher melting point than gangaleoidin, but that the amount was too small to allow of examination; that no sugar alcohol was isolated, but that a water-soluble ester or lactone appeared to be present. The more complete investigation of the lichen referred to in the earlier paper has now been carried out. We confirm that the main constituent is gangaleoidin and that atranorin and chloratranorin are present, the latter forming about 80 per cent. of the mixture of these two bodies. The substance with a higher melting point than gangaleoidin we find to melt at 231–233° C. (gangaleoidin m.p. 213–214° C.). Analyses show that its carbon, hydrogen, and chlorine content are practically identical with those of gangaleoidin, but three analyses for methoxyl gave 5·17 per cent., 7·9 per cent., and 8·0 per cent., as against 15 per cent. for gangaleoidin, the methoxyl being split off with great difficulty; the actual presence of methoxyl is an open question. The substance does not behave like a depsidone, a lactone linking not being opened with the extreme ease characteristic of bodies of the latter type. For the substance we suggest, with reserve, the formula $C_{26}H_{21}O_{10}Cl_3$. We are unable to confirm the presence of a water-soluble ester or lactone, but, on the other hand, have isolated arabitol. We have also found in the lichen small amounts of the coloured products endococcin and rhodophyscin. We have made the interesting observation that endococcin is converted into rhodophyscin on short boiling

with acetic acid. The two bodies are obviously closely related and may be isomeric, but the analyses are not conclusive on this latter point. Rhodophyscin does not appear to be an anthraquinone. In testing for an ortho-quinone by allowing some of the material to stand in acetic acid solution with o-phenylene-diamine, no precipitate of an azine was formed even on dilution with water. Furthermore this solution no longer gives with alkali the characteristic colour reactions of endococcin or rhodophyscin. The insolubility of rhodophyscin in sodium bisulphite supports the view that it is not a phenanthrone. The reactions of the two bodies rather recall the behaviour of the natural hydroxy-naphthaquinones, e.g. lapachone or dunnione (Robinson and Price, J.C.S., 1939, p. 1522). Owing to the insolubility of rhodophyscin or its acetate in camphor, a molecular weight was not determined, but the empirical formula is $(C_3H_2O)_n$, and n is probably either 5 or 6; for a formula $C_{15}H_{10}O_5$ there are three hydroxyl groups present and for $C_{18}H_{12}O_6$ four. Rhodophyscin contains no methoxyl. We have confirmed the formula $C_{18}H_{14}O_7Cl_2$ for gangaleoidin given in the earlier paper, but the substituents in the skeleton formula have to be revised. Gangaleoidin contains two methoxyl groups; on treatment with diazomethane a monomethyl derivative is formed, so that the formula may be written $C_{16}H_7O_4Cl_2(OCH_3)_2(OH)$. With methyl alcoholic potash one molecule of methyl alcohol is added on, and the resulting product is soluble in sodium hydroxide, but insoluble in sodium carbonate. This indicates the presence of a lactone group in gangaleoidin. Treatment of the opened product with diazo-methane gives a product containing five methoxyl groups. Thus the formula may be written $C_{15}H_7O_2Cl_2(COO)(OCH_3)_2(OH)$. When the pentamethoxy compound is hydrolysed by methyl alcoholic potash, two methyl groups are split off, and the resulting compound is a dicarboxylic acid, from which one carboxyl group is eliminated readily on heating in formic acid. Consequently we can now write the formula

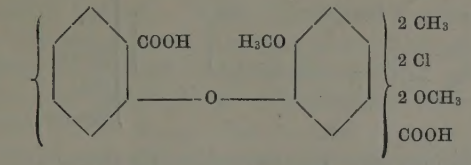
$$C_{14}H_7OCl_2(COO)(COOCH_3)(OCH_3)(OH).$$

General considerations of this formula and lack of reactivity of the remaining oxygen atom indicate the presence of two aromatic rings joined by an oxygen bridge. Since the chlorine atoms are not affected by boiling alcoholic potash they cannot be present in an aliphatic side chain, but must be directly attached to the benzene ring. General considerations of lichen chemistry favour the view that the aliphatic side chains consist of two methyl groups, and not of a single ethyl substituent. Accordingly we may write the formula,



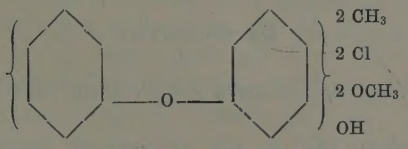
I.

We have attempted, by means of pyrolytic methods, to effect scission of the molecule, but so far without success. When the compound,



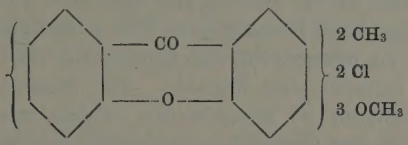
II.

was distilled under reduced pressure three compounds were obtained; one of these was the monocarboxylic acid produced by partial decarboxylation; the second was a diphenyl ether of the constitution



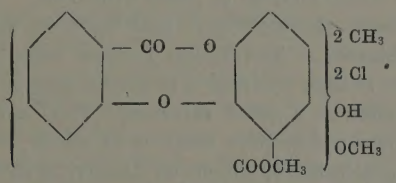
III.

complete decarboxylation and partial demethylation having taken place; the third was a neutral product of formula $C_{18}H_{16}O_5Cl_2$, which contains three methoxyl groups. This product may be a xanthone,



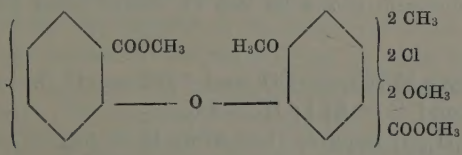
IV.

Should this product have been formed without the occurrence of any group wanderings, then the carboxylic ester group in gangaleoidin must be in the ortho-position to the oxygen bridge as in V—



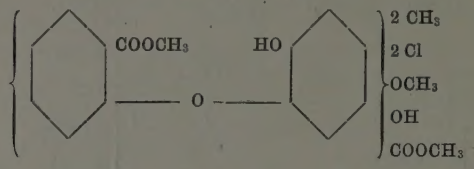
V.

or, alternatively, this position must be unsubstituted. However, the absence of group wandering must be accepted with reserve, for, whereas the compound



VI.

may be distilled unchanged, the compound



VII.

under similar conditions gives rise to an isomeric compound.

Attempts to effect dechlorination or fission by heating with hydriodic acid gave unworkable products.

Experimental.

Extraction of Lecanora gangaleoides *with Ether.*

The air-dried lichen, 1370 g., was exhaustively extracted with ether and the extract yielded 95 g. of a yellowish solid.

The solid was treated with two successive amounts (250 c.c.) of warm ligroin, and filtered. The filtrate was evaporated, and the residue, a brown oily solid, was warmed with 50 c.c. alcohol, and filtered. The portion insoluble in alcohol on crystallising from benzene gave 0·65 g. atranorin. On evaporating the alcohol solution an orange-coloured tarry solid was obtained, which was boiled with 20 c.c. benzene, and filtered. The benzene solution on cooling deposited a small amount of gangaleoidin, which was recrystallised from xylene; the benzene mother liquors on evaporation gave a product which, after a couple of crystallisations, gave white needles melting at 231–233° C. The portion of the orange-coloured solid insoluble in benzene was washed with 20 c.c. ether, and then with 15 c.c. acetone. To the acetone solution was added 15 c.c. methyl alcohol, and the whole allowed to stand overnight. Yellow needles were precipitated. These were collected and recrystallised from acetone-methyl alcohol; 6 mg. were obtained. The yellow needles were insoluble in sodium carbonate, but soluble in sodium hydroxide to give a purple-red solution. They dissolved in sulphuric acid to a green solution. They consist of endococcin. On boiling with acetic acid the solution deepens in colour, and on dilution with water a red powder is precipitated, which, on dissolving in acetone and adding methyl alcohol, is deposited in the characteristic red crystalline form of rhodophyscin. These crystals give with sulphuric acid the colour reactions of rhodophyscin. Endococcin shows no loss in weight when heated in vacuum at 50° C.

4·362 mg. gave 10·735 mg. CO_2 and 1·570 mg. H_2O.

Found C = 67·1; H = 4·0.

$C_{16}H_{12}O_5$ requires C = 67·6; H = 4·2.

$C_{15}H_{10}O_5$ requires C = 66·6; H = 3·7.

There was not sufficient material for a methoxyl determination. The portion of the ligroin-soluble material which was insoluble in benzene, ether, and acetone, was a reddish powder. It was dissolved in sufficient acetone for the purpose, and two volumes of methyl alcohol added. On standing in an open dish overnight, deep red crystals had separated. These were filtered, washed with methyl alcohol, and dried; 15 mg. were obtained. The product is soluble in sodium carbonate and in caustic soda to form a purple red solution. It dissolves in sulphuric acid to a raspberry-red solution which soon becomes permanently green like the solution of endococcin in sulphuric acid. It gives no colour reaction with ferric chloride. It is rhodophyscin.

5·162 mg. dried in high vacuum at 50° C. lost 0·282 mg.
4·880 mg. dried substance gave 11·900 mg. CO_2 and 1·580 mg. H_2O.
Found $H_2O = 5{\cdot}46$; $C = 66{\cdot}5$; $H = 3{\cdot}6$.
$C_{15}H_{10}O_5$, H_2O requires $H_2O = 6{\cdot}25$; $C_{18}H_{12}O_6$, H_2O requires $H_2O = 5{\cdot}26$.
$C_{15}H_{10}O_5$ or $C_{18}H_{12}O_6$ requires $C = 66{\cdot}6$; $H = 3{\cdot}7$.

Rhodophyscin contains no methoxyl. The acetate was prepared by dissolving the substance in acetic anhydride, adding a couple of drops of pyridine, and boiling for two minutes. The light yellow solution was precipitated with water, the precipitate collected and crystallised from dilute acetic acid, from which it separated as yellow needles. With sulphuric acid it gives the same colour reactions as rhodophyscin; it gives no colour reaction with sodium carbonate or caustic soda.

2·124 mg. gave 4·970 mg. CO_2 and 0·770 mg. H_2O.
Found $C = 63{\cdot}34$; $H = 4{\cdot}03$.
$C_{15}H_{10}O_5 . 3CH_2CO$ requires $C = 63{\cdot}6$; $H = 4{\cdot}03$.
$C_{18}H_{12}O_6 . 4CH_2CO$ requires $C = 63{\cdot}4$; $H = 4{\cdot}06$.

Both rhodophyscin and its acetate are insoluble in camphor.

To a solution of rhodophyscin in acetic acid was added some o-phenylene-diamine; on prolonged standing there was no separation of material, even on dilution with water. The solution on being made alkaline with caustic soda did not give the characteristic colour reaction of rhodophyscin. Control experiments with various trihydroxy-anthraquinones showed that the presence of the diamine does not affect their colour reactions. When the reaction was carried out in boiling acetic acid a dark amorphous precipitate was formed, possibly an oxidation product of the diamine.

The portion of the ether-extract of the lichen which was insoluble in ligroin was treated with two successive amounts of 25 c.c. of warm benzene, and filtered. The insoluble portion was mainly gangaleoidin, and was purified by crystallisation from xylene. The benzene mother liquors were allowed to stand, and were then filtered from a deposit, which on crystallisation from xylene was found to be gangaleoidin. The total yield was 30 g.

The benzene mother liquors were evaporated, and the red solid which remained was treated successively with 50 c.c. ether, 25 c.c. warm ligroin, and 25 c.c. warm benzene. The residue was warmed with 15 c.c. chloroform, and filtered. From the chloroform solution was obtained 0·2 g. atranorin. The portion insoluble in chloroform was crystallised from 8 c.c. xylene, and gave 0·15 g. of a substance melting at 231–233° C.

5·036 mg. gave 9·650 mg. CO_2. and 1·590 mg. H_2O.
5·309 mg. gave 10·210 mg. CO_2. and 1·680 mg. H_2O.
9·147 mg. gave 6·310 mg. AgCl; 9·101 mg. gave 6·240 mg. AgCl.
3·193 mg. gave 1·270 mg. AgI.
2·761 mg. gave 1·650 mg. AgI; 2·418 mg. gave 1·470 mg. AgI.
Found C = 52·26; 52·45. H = 3·51; 3·51. Cl = 17·07; 16·95.
OCH_3 = 5·17; 7·90; 8·03.
$C_{26}H_{21}O_{10}Cl_3$ requires C = 52·04; H = 3·50; Cl = 17·7.

Schoeller states that the methoxyl is split off with difficulty and the determination takes a long time.

0·289 mg. in 4·314 mg. camphor, 4·0 △. M.W. 670.
$C_{26}H_{21}O_{10}Cl_3$ requires M.W. 599·5.

The substance is extracted with difficulty from solution in ether by sodium bicarbonate; readily by sodium carbonate. It forms a colourless solution in caustic soda. It gives no colour reaction with bleaching powder; with ferric chloride a light purple, and with sulphuric acid a pale yellow.

A solution of 0·1 g. of the substance in acetone was methylated with an ether solution of diazomethane. After standing overnight, the solvent was evaporated, and the residue crystallised from 3 c.c. ligroin. The methyl ether separated as large prisms m.p. 143–144° C.

4·149 mg. gave 8·250 mg. CO_2; and 1·590 mg. H_2O.
3·837 mg. gave 4·390 mg. AgI.
Found C = 54·23; H = 4·26; OCH_3 = 15·1.
$C_{26}H_{18}O_7Cl_3(OCH_3)_3$ requires C = 54·25; H = 4·21; OCH_3 = 14·5.

Acetone Extract of the Lichen.

After the lichen had been extracted with ether it was extracted with boiling acetone. The acetone solution on evaporation left a dark resinous solid. This was boiled with 100 c.c. benzene and filtered. From the benzene solution, on cooling, a product was obtained which, on crystallising from a mixture of chloroform and alcohol, proved to be atranorin. Weight 1·4 g.

The portion insoluble in benzene was boiled with 100 c.c. alcohol and filtered. The alcohol solution was evaporated, the residue extracted with water, and the portion soluble in water recovered. This was then dissolved in the

minimum quantity of hot alcohol, decolorised with charcoal, and to the solution an equal volume of ligroin added. On standing crystals of arabitol were deposited. Weight 1·3 g., m.p. 101° C.

3·727 mg. gave 5·325 mg. CO_2; 2·690 mg. H_2O.
Found C = 38·97; H = 8·02.
$C_5H_{12}O_5$ requires C = 39·47; H = 7·90.

0·1 g. of the compound was boiled for 30 min. with 0·1 g. sodium acetate and 0·5 c.c. acetic anhydride. The mixture was then poured into water; the substance which separated was dried and crystallised from ligroin. The lustrous crystals melted at 74–75° C. A mixed melt with d-arabitol acetate showed no depression.

The portion of the acetone extract insoluble in the benzene and alcohol was extracted with chloroform. The extract on crystallisation from a mixture of chloroform and alcohol gave a further 1·2 g. of atranorin.

Alcohol Extract of the Lichen.

The lichen after extraction with ether and acetone was finally extracted with boiling alcohol. On evaporating the extract a brown oily solid was obtained. A benzene extract of a portion of this material showed on evaporation a fungus-like growth of crystals. Portion of the main extract was distilled with steam. The distillate, which on cooling deposited crystals, was extracted with ether and the extract crystallised from ligroin. β-Orsellinic methyl ester was obtained, m.p. 144° C. This was presumably formed by the breakdown of atranorin during the extraction.

2·638 mg. gave 5·935 mg. CO_2; 1·520 mg. H_2O.
2·679 mg. gave 3·060 mg. AgI.
Found C = 61·36; H = 6·4; OCH_3 = 15·07.
$C_9H_9O_3(OCH_3)$ requires C = 61·22; H = 6·12; OCH_3 = 15·8.

The second portion of the main extract was boiled with acetone. The insoluble residue was extracted with water, the aqueous extract recovered, boiled with alcohol and filtered. The filtrate was decolorised with charcoal, and to the filtered solution an equal volume of ligroin added. On standing the solution deposited crystals of d-arabitol, m.p. 101° C.

INVESTIGATION OF GANGALEOIDIN.

Gangaleoidin methyl ether.

0·35 g. gangaleoidin was dissolved in 25 c.c. acetone, and to the solution an ether solution of diazomethane added. After standing, the solvent was

evaporated, and the residue crystallised from a mixture of 15 c.c. benzene and 20 c.c. ligroin. The crystals were rectangular prisms, m.p. 181° C.

4·933 mg. gave 9·695 mg. CO_2; 1·690 mg. H_2O.
8·258 mg. gave 5·505 mg. AgCl; 3·611 mg. gave 6·055 mg. AgI.
Found C = 53·6; H = 3·81; Cl = 16·5; OCH_3 = 22·1.
$C_{16}H_7O_4Cl_2(OCH_3)_3$ requires C = 53·4; H = 3·75; Cl = 16·6; OCH_3 = 21·8.

Methyl ester from Gangaleoidin.

This compound was prepared by the method described in Part I (p. 144). It melted at 198·5° C. Two grams were distilled under reduced pressure in four portions of 0·5 g. each. The distillate was heated with 5 c.c. ligroin, and the insoluble portion crystallised twice from a mixture of 5 c.c. benzene and 5 c.c. ligroin, m.p. 184–185° C. A mixture with the original material melted at 166° C.

4·717 mg. gave 8·825 mg. CO_2; 1·690 mg. H_2O.
3·488 mg. gave 5·330 mg. AgI; 8·669 mg. gave 5·490 mg. AgCl.
Found C = 51·03; H = 3·98; Cl = 15·7; OCH_3 = 20·2.
$C_{16}H_9O_5Cl_2(OCH_3)_3$ requires C = 51·24; H = 4·04; Cl = 16·0; OCH_3 = 20·9.
0·281 mg. in 4·007 mg. camphor gave 5·6° △; 0·270 mg. in 6·059 mg. camphor gave 3·5° △.

Found M.W. 501; 509. $C_{19}H_{18}O_8Cl_2$ requires 445.

Isomerisation has thus taken place on distilling the methyl ester.

Methylester methylether from Gangaleoidin.

A mixture of 0·15 g. gangaleoidin methylether and 3 c.c. of a 5 per cent. solution of methyl alcoholic potash was heated on the water bath for one minute. The solution was then diluted with water and acidified. The solid formed was crystallised from a mixture of 7·5 c.c. benzene and 15 c.c. ligroin, forming prisms, m.p. 186–187° C.

4·839 mg. gave 9·320 mg. CO_2; 1·850 mg. H_2O.
Found C = 52·56; H = 4·25; $C_{16}H_8O_4Cl_2(OCH_3)_4$ requires C = 52·29; H = 4·36.

Methylester dimethylether from Gangaleoidin.

This compound has been got by methylating with diazomethane the substance m.p. 187° C. referred to in the previous paragraph, or, more conveniently, by methylating with diazomethane the methyl ester, m.p. 198° C., obtained by the action of methyl alcoholic potash on gangaleoidin. The product crystallised

from a mixture of three parts of benzene and five parts of ligroin; m.p. 141–142° C.

4·870 mg. gave 9·515 mg. CO_2; 2·070 mg. H_2O.
8·860 mg. gave 5·260 mg. AgCl; 3·850 mg. gave 9·345 mg. AgI.
Found C = 53·3; H = 4·72; Cl = 14·7; OCH_3 = 32·0.
$C_{16}H_7O_3Cl_2(OCH_3)_5$ requires C = 53·2; H = 4·65; Cl = 15·0; OCH_3 = 32·8.

Hydrolysis of Gangaleoidin.

When gangaleoidin is heated for three hours on the water bath with 15 per cent. methyl alcoholic potash the only product formed is the methyl ester, m.p. 198° C. Partial hydrolysis takes place under the following conditions:—1 g. gangaleoidin is boiled with 100 c.c. 15 per cent. methyl alcoholic potash for five hours. The greater part of the alcohol is then distilled off, 50 c.c. water added and the remainder of the alcohol removed, and a further 50 c.c. water added. The solution is then acidified, extracted with ether, and the yellow gum recovered from the ether. This gum is treated with 10 c.c. benzene, and then dissolved in ether. On slow evaporation of the ether, crystals separated, which were recrystallised from a mixture of xylene and alcohol. The crystals are hydrated, and lose their water of crystallisation with difficulty. The monohydrate has m.p. 197–198° C.; the dihydrate m.p. 161° C.

Substance m.p. 197–198° C.

4·394 mg. gave 7·805 mg. CO_2; 1·620 mg. H_2O.
14·828 mg. gave 8·850 mg. AgCl; 2·790 mg. gave 2·820 mg. AgI.
Found C = 48·44; H = 4·10; Cl = 14·75; OCH_3 = 13·36.
$C_{16}H_{10}O_6Cl_2(OCH_3)_2 . H_2O$ requires C = 48·1; H = 4·0; Cl = 15·8; OCH_3 = 13·8.

Substance m.p. 161° C.

5·129 mg. gave 8·715 mg. CO_2; 1·970 mg. H_2O.
17·750 mg. gave 11·030 mg. AgCl; 3·731 mg. gave 2·850 mg. AgI.
Found C = 46·34; H = 4·27; Cl = 15·37; OCH_3 = 11·2.
$C_{16}H_{10}O_6Cl_2(OCH_3)_2 . 2H_2O$ requires C = 46·2; H = 4·3; Cl = 15·2; OCH_3 = 13·2.

On treatment with diazomethane these products gave the methylester dimethylether, m.p. 141–142° C.

Hydrolysis of Gangaleoidin methylester dimethylether.

0·2 g. of the methylester, m.p. 141–142° C., was boiled with 20 c.c. 15 per cent. methyl alcoholic potash for four hours. The alcohol was then removed, the solution diluted with water, acidified, and the precipitate crystallised from

50 per cent. alcohol. The acid thus obtained consisted of white prisms, m.p. 216–217° C.

4·819 mg. gave 8·800 mg. CO_2; 1·860 mg. H_2O.
13·306 mg. gave 8·290 mg. AgCl; 3·578 mg. gave 5·475 mg. AgI.
Found C = 49·8; H = 4·3; Cl = 15·4; OCH_3 = 20·2.
$C_{14}H_7OCl_2(COOH)_2(OCH_3)_3 . H_2O$ requires C = 49·2; H = 4·3; Cl = 15·3; OCH_3 = 20·1.

0·5 g. of the above dicarboxylic acid was heated in 15 c.c. formic acid for 30 min. The solution was then diluted, and the solid which was precipitated crystallised from ligroin, giving white prisms, m.p. 138–139° C. The same product is obtained when the dicarboxylic acid is heated just above its melting point, but not when heated in acetic acid or acetic anhydride, the material being recovered unchanged from these solvents.

4·704 mg. gave 9·315 mg. CO_2; 2·000 mg. H_2O.
6·252 mg. gave 4·390 mg. AgCl; 2·648 mg. gave 4·550 mg. AgI.
Found C = 54·01; H = 4·72; Cl = 17·8; OCH_3 = 22·66.
$C_{14}H_8OCl_2(COOH)(OCH_3)_3$ requires C = 53·9; H = 4·5; Cl = 17·7; OCH_3 = 23·2.

0·1 g. of the above monocarboxylic acid dissolved in acetone was methylated with an ether solution of diazomethane. On evaporation of the solvent the residue was crystallised from methyl alcohol, giving rectangular crystals, m.p. 79–80° C.

4·632 mg. gave 9·360 mg. CO_2; 2·000 mg. H_2O.
10·136 mg. gave 7·135 mg. AgCl; 3·570 mg. gave 8·000 mg. AgI.
Found C = 55·13; H = 4·8; Cl = 17·6; OCH_3 = 29·6.
$C_{14}H_8OCl_2(COOCH_3)(OCH_3)_3$ requires C = 54·94; H = 4·82; Cl = 17·1; OCH_3 = 29·9.

Pyrolysis of Dicarboxylic acid, m.p. 216–217° C.

I. *In glycerine.*—0·5 g. of the acid was heated in 10 c.c. glycerine for 5 hours at 220–225° C. The whole was then diluted with 100 c.c. water, and the solid collected. This was dissolved in ether, and extracted with 2 per cent. sodium carbonate.

The sodium carbonate layer (B) was acidified and extracted with ether; the product recovered from the ether was crystallised from ligroin, giving crystals, m.p. 137–139° C., identical with the monocarboxylic acid referred to above.

The ether layer (A) gave a residue, which was first crystallised from 50 per cent. alcohol and then from ligroin. The product had m.p. 165–166° C. and was soluble in sodium hydroxide.

4·921 mg. gave 10·080 mg. CO_2; 2·100 mg. H_2O.
6·230 mg. gave 5·210 mg. AgCl; 3·769 mg. gave 5·270 mg. AgI.
Found C = 55·86; H = 4·74; Cl = 20·7; OCH_3 = 18·4.
$C_{14}H_9OCl_2(OH)(OCH_3)_2$ requires C = 55·98; H = 4·66; Cl = 20·8; OCH_3 = 18·1.

The above phenol was methylated in acetone solution with diazomethane, and the white residue recovered from the solvent, crystallised from ligroin, giving prisms, m.p. 112–113° C.

5·020 mg. gave 10·510 mg. CO_2; 2·310 mg. H_2O.
Found C = 57·10; H = 5·11.
$C_{14}H_9OCl_2(OCH_3)_3$ requires C = 57·14; H = 5·04.

II. *By distillation.*—Two grams of the dicarboxylic acid were distilled under reduced pressure in four portions. The brown oily distillate was dissolved in chloroform, and the solution extracted with 3 per cent. sodium carbonate. The brown upper layer, which showed a marked olive-green fluorescence, was acidified and extracted with chloroform; from the chloroform a light brown oil (A) was recovered.

The chloroform layer containing material insoluble in sodium carbonate was extracted with 3 per cent. potash. The light red upper layer was acidified and extracted with chloroform; from the chloroform a brown solid (B) was recovered.

From the chloroform solution left after treatment with sodium carbonate and potash a light brown oily solid was obtained (C).

The product A was dissolved in 5 c.c. methyl alcohol, and to the warm solution 2·5 c.c. water added. On standing white prisms were deposited, m.p. 139° C., identical with the monocarboxylic acid referred to above.

The product B was crystallised from ligroin, and gave a substance, m.p. 166° C., identical with the phenol obtained on heating the dicarboxylic acid in glycerine.

The product C was washed with 5 c.c. warm ligroin, and the insoluble portion crystallised from ethyl alcohol, giving hexagonal crystals, m.p. 212–213° C.

4·826 mg. gave 10·015 mg. CO_2; 1·830 mg. H_2O.
5·661 mg. gave 4·360 mg. AgCl; 4·818 mg. gave 8·980 mg. AgI.
Found C = 56·6; H = 4·21; Cl = 19·05; OCH_3 = 24·6.
$C_{15}H_7O_2Cl_2(OCH_3)_3$ requires C = 56·4; H = 4·18; Cl = 18·5; OCH_3 = 24·3.
0·202 mg. in 2·899 mg. camphor gave 7·7° $\triangle$; M.W. 362.
$C_{18}H_{16}O_5Cl_2$ requires 383.

We wish to acknowledge the valuable assistance of M. E. Mohan; M. A. Callan; E. J. Kennedy; and M. Smyth during the course of the experimental work.

No. 20.

FURTHER EXPERIMENTS ON TRANSPIRATION INTO A SATURATED ATMOSPHERE.

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(Plate 5.)

[Read December 19, 1939. Published separately February 22, 1940.]

When the Cohesion Theory of the Ascent of Sap was formulated the possibility of accounting for transpiration by simple evaporation from water-surfaces held in the imbibed substance of the evaporating cells was carefully considered. It was easy to devise a lifeless model of a tree, composed of inanimate material only, capable of raising water continuously from a moist soil, transmitting it by means of tensile columns, and setting it free from evaporating surfaces supported in porous material (1).

Several considerations even then suggested that this was not a complete description of the process in the living plant. It is a matter of common observation that dead leaves (killed by steam, poisonous vapours, or other means) cease to draw up sufficient water to maintain their imbibed state, and soon become dry and crisp. Living leaves on the same branches, although also giving off water vapour, remain filled with water, moist and sappy. This strongly suggests the possibility that the cells supporting the evaporating surfaces of water act as machines raising the water and exposing it for evaporation on their outer surfaces—or, perhaps it may be preferable to regard them as glands secreting water on their outer surfaces.

It was also evident that, if transpiration in the plant is essentially similar to the process occurring in the inanimate physical model just imagined, a cut shoot, set upright in a vessel of water surrounded by a water-saturated space, should allow the continuous passage of water through the leaf-tissues and down the stem; for condensation would occur on the water surfaces in the cell-walls of the leaves, rendered re-entrant by the weight of the water columns hanging from them. Actually when dead and living cut shoots are placed side by side in the same saturated space, the living continue to draw up water, while no rise can be detected in the dead. The rise in the living may be made visible by adding a dye (eosin) to the water below. Of course in these experiments care must be taken that the shoots used are fully glutted with water before the dye is applied. Otherwise water entering to make good any deficiency might carry the eosin into the shoot and upwards in the stem. A suspicion that this

precaution had not received full attention made Smith, Dustman, and Shull question Dixon's early results (3).

Living shoots completely submerged also show this ability of raising water from a lower to a higher level. Furthermore it was soon found that when water free from dissolved gases surrounded the leaves, the phenomenon was brought to a stand-still. When oxygen is generated in the leaf-cells by photosynthesis, shoots submerged even in boiled water to which carbon dioxide has been added, will draw up water when exposed to light. All these early observations suggested that the work of raising water in subaqueous transpiration is done at the expense of respiratory energy (1).

The possibility that the observed rise of eosin solution might be due to a water-deficit in the experimental branch was fully investigated before the first publication on this subaqueous transpiration, and conclusive results were recorded of water-logged shoots and leaves raising eosin solution to considerable heights (1 and 2). A few more striking experiments recently made on this subject may be here described:—

A shoot of *Ribes sanguineum* about 26 cm. long with 6 mature and 2 smaller leaves, severed from its plant under boiled water, was submerged completely under aerated water for 5 hours. During this time, with the object of removing gas bubbles, rarefied or other, in the tracheae, several changes of cooled boiled water were presented to the cut stem. At v.00 p.m. an eosin solution was applied to the cut surface of the stem, while the upper part of the stem with its leaves was still submerged in water, kept aerated by a stream of air bubbles. Next day at xi.00 a.m. eosin was found 15 c.m. above the level of the applied solution.

A shoot of *Buddleia globosa* 40 cm. long and carrying 6 large leaves was treated similarly at the same time. At xi. 15 a.m. on the second day the eosin was found in the pair of leaves springing from the node 32 cm. above the level of the applied eosin.

On the following day (July 5th) a shoot of *Ribes sanguineum* 27 cm. long with 7 leaves, and a coiled branch of *Humulus lupulus* (golden) 69 cm. long with 3 pairs of leaves, were cut under water from the parent plants, and completely submerged under aerated water for 4 hours. With their foliage and stems still submerged they were then supplied through their cut surfaces with eosin solution. Inspection on the next day, 19 hours after the application of the eosin, showed the tracheae of the Ribes completely injected throughout the shoot, even to the outermost extremities of the veins of the highest leaves. The Humulus shoot showed injection of the veins of the highest expanded leaves, i.e. those of the node 55 cm. from the lower end. A few bundles were injected in the internode above this—68–69 cm. from the eosin supply.

A branch of *Leycesteria formosa* 36 cm. long with 9 leaves, similarly treated, was also completely injected with eosin to the very tips. Again, a shoot of *Quercus robur* 28 cm. long with 5 short lateral branches, and carrying 50 leaves in all, gave a similar result under similar treatment.

In contrast to these was a branch of *Ribes sanguineum* 22 cm. long with

5 mature leaves, which, after cutting and similar submersion, was plunged in water near the boiling point for about 20 sec. It too was surrounded with aerated water and supplied with cooled boiled water below. The latter was replaced 4 hours subsequently with eosin solution. 19½ hours later no significant rise of eosin in the stem was registered.

In several experiments no rise of eosin solution in the stem, or only a very slight one, was found when the leaves were surrounded with cooled boiled water. Similarly, cold boiled water containing dissolved carbon dioxide surrounding the leaves prevented the rise of eosin solution in the tracheal paths of the stems and leaves as long as the leaves were screened from light. It was found, however, that illumination strong enough to produce sensible photosynthesis in the last-mentioned conditions, enabled the shoot to raise eosin solution in its woody tissues.

These and similar observations render it very probable that respiratory processes supply the leaf-cells with energy to secrete water drawn from the tracheae into the leaf spaces.

Further evidence on this point of view was sought by the experimental comparison of the rate of transpiration into saturated spaces when free oxygen is available to the leaf-cells, and when it is excluded by the presence of nitrogen.

To make this comparison a leafy shoot, fixed in a potometer (B, Plate 5, fig. 1) having a horizontal index-tube, was surrounded with a glass bell (A). Arrangements were made to keep the space within the bell saturated by lining it with bibulous paper dipping below into the water which lay in the lower part of the bell. The bell was closed above by a glass plate having a trapped exit tube (H) dipping into water in a small beaker. The neck of the bell below was closed by a rubber bung furnished with two perforations. Through the smaller of these a gas-supply pipe entered, and the larger held tightly the upper end of the potometer. The gas-supply pipe by means of a two-way tap could deliver a stream of air or of nitrogen into the bell. Fig. 1 shows the electric air pump (D) connected. The source of nitrogen was a cylinder of the compressed gas provided with a regulating tap. Before the gas stream was led into the bell it was bubbled through water in the vessel (E). The apparatus was set up in a dark room, artificial light being used for making the readings. The first experiments were made using an index-tube having a fine bore of elliptical section 0·54 × 0·70 mm. in the hope that it would give a large movement of the index for a small amount of transpiration. Also the gas was supplied very slowly in order that the chamber containing the experimental branch would remain fully saturated. The readings, however, were fluctuating and unsatisfactory. These faults were attributed to the sticking of the bubble in the narrow index-tube of the potometer, and to the slowness of the replacement of the air in the bell by nitrogen. The first was obviated by using a wider index tube of circular bore 1 mm. in diameter. In order to be certain that the glass bell remained quite saturated during the experiment, in the preliminary arrangement it was deemed necessary that the gas-current should be extremely slow. To effect this the pump and the gas-cylinder tap were

adjusted to deliver 8 c.c. per minute. The capacity of the bell or leaf-chamber was 5 litres. Hence at this rate of supply it would require more than 10 hours to introduce a volume of nitrogen equal to the volume of the leaf-chamber. In order to be able to speed up substantially the flow of gas without the fear of changing its state of saturation, it was arranged to supersaturate the gas stream by raising the temperature of the bubbling flask (E) considerably (say 25° C.) above that of the leaf-chamber (A). In this way constant supersaturation round the transpiring leaves, and continuous condensation of water on their surfaces, were maintained, even when the rate of flow was raised 3 or 4 times. Allowing for the increased loss of nitrogen this should reduce the percentage of oxygen at the end of 2½ to 3½ hours to about 7% as compared to 21% in the atmosphere.

To fix the branch hermetically in the potometer, a tightly fitting pliant rubber collar was put on the lower part of the experimental stem below its leaves and 8–10 cm. from its cut end. To effect this without injuring the bark of the stem a 2 cm. length of soft black rubber tube lubricated with glycerine was forced on to the end of a thin brass tube into which the stem fitted loosely. This brass tube was then brought up round the stem covering its lowermost 10 cm., and the rubber tube slipped from it on to the branch. By a similar manœuvre with a slightly larger brass tube a rubber bung is slipped on to the black rubber collar. The bung, now holding the branch, is firmly pressed into the potometer tube, which has been filled with water. This method secures a completely gas-tight connection between the branch and the potometer tube.

With these arrangements on October 23rd a branch of *Sambucus nigra*, grown in the open, carrying 5 pairs of leaves was cut from its parent plant under cooled boiled water. Still supplied with tepid boiled water it was submerged under tepid water for one hour. During this time while the tepid surrounding water was cooling slowly the branch was fitted with its rubber collar and bung, and a fresh surface was cut on the lower end. The branch was then put down into the glass bell, or leaf-chamber, and the bung clasping its lower end pressed into the potometer tube. Then the larger bung carrying the gas-supply tube and the potometer tube was pressed tightly up into the neck of the glass bell. A gasket of bibulous paper on the upper rim of the bell, the lining of bibulous paper, and the branch, were liberally sprayed with water. The bell was closed with the glass plate carrying the exit tube dipping into the beaker (H) of water. A second beaker (I) was also arranged at the level of the rim, supplying, across a bridge of bibulous paper, water to the gasket between the glass plate and the rim of the bell. When these adjustments were completed the electric air-pump was started, and the stream of supersaturated air began to pass from the flask E into the bell, and to issue through the trapped tube at H. A thermometer in A showed that the temperature in the leaf-chamber was 23° C. Accordingly the height of E above the electric hot plate F was adjusted so that the temperature shown by a thermometer immersed in the water in E remained about 48° C. During the time of observation these temperatures did not fluctuate more than 3° C. It may be noticed that as this

temperature change was due to the daily heating up of the laboratory it had the form of a steady rise from morning till late afternoon. Hence the thermal expansion of the water in the potometer would, if significant, act against transpiration from the leaves. After the apparatus had been set up, and the supersaturated air had been flowing through it for 20 hours, a single bubble was introduced into the index-tube and its rate of movement determined by successive readings of the vernier microscope L. From x. 40 a.m. to xii. 20 p.m. the rate of movement, beginning at 0·666 mm. per min., steadied at 1·100 mm. Supersaturated nitrogen was then introduced, and the rate from xii. 20 to ii. 16 averaged 1·110. From ii. 16 to iii. 0 p.m. it was 0·675; at iii. 06 it had fallen to 0·050 mm./min., and from iii. 16 to iii. 36 it was zero. An air current was then bubbled through, and at iv.04 the rate became 1·700, at iv. 54 2·100, and at vi. 00 1·390 mm./min. On the next day, October 25th, at noon the rate of index-movement in supersaturated air had fallen to 0·380 mm./min.

Fig. 2 graphically represents the observations of this experiment.

Fig. 3 records a similar experiment on a similar branch of *Sambucus nigra* similarly treated. On October 26th from xi. 16 a.m.–xii. 06 p.m., the branch being surrounded with supersaturated air, the rate was about 1·630 mm./min. At xii. 06 p.m. supersaturated nitrogen was supplied, the rate fluctuating from 1·200 to 2·150 averaged 1·690 between xii. 06 and i. 47 p.m. It then quickly fell from 1·050 mm. at i. 57 p.m. to 0·450 at ii. 27 p.m. At ii. 32 p.m. movement ceased. At iii. 00 p.m. supersaturated air was supplied, but up to v. 30 p.m. no movement could be detected. From v. 30–vi. 31 p.m. only 1·300 mm. movement was registered. The supply of supersaturated air was kept up during

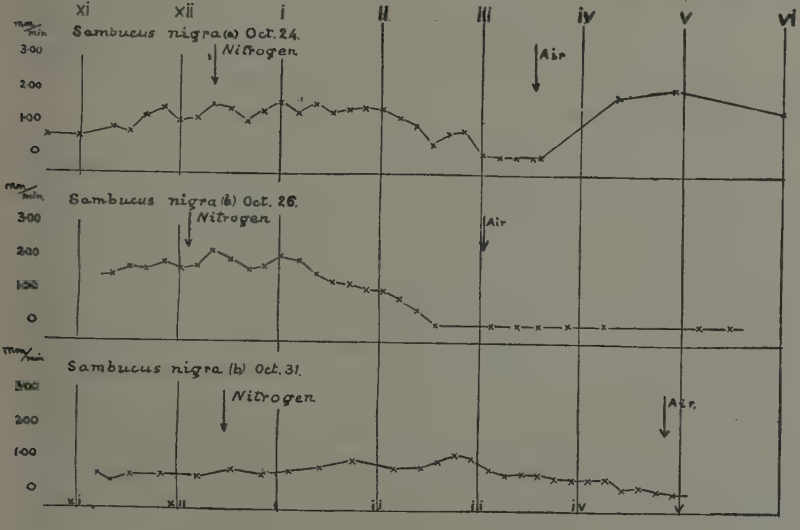

FIG. 2

FIG. 3

FIG. 4

the night, but next morning, October 27th, the movement of the index was still very sluggish. At noon there was an improvement, and the average rate from xii. 03 to ii. 35 p.m. was 0·550 mm./min. Between iii. and vi. 30 it quickened to 1·133, but next morning, October 28th, had ceased. When the branch was taken out of the apparatus, several of the leaflets were blotched and injected with water. A few had been shed.

With similar preparatory treatment on October 30th a shoot of *Sambucus nigra* gave in supersaturated air on the next day between xi. 00 a.m. and xii. 25 p.m. an average index-movement of 0·422 mm./min. Supersaturated nitrogen was then supplied and continued till iv. 51 p.m. With a preliminary fluctuation to 1·150 mm./min. at iii. 00 p.m. the rate finally fell to zero at iv. 50 p.m. (Fig. 4). Then supersaturated air was supplied and continued through the night. Next day, Nov. 1st, the rate rose to 0·544 mm./min. in the afternoon, but on November 2nd fell again. When taken from the apparatus the shoot was found to have shed about half of its pinnae, but all still retained a turgid and fresh appearance.

The next subject for experiment was a branch cut from a conservatory specimen of *Calceolaria burbidgei* with six small lateral branches and carrying 32 leaves and some flowers. It was cut under boiled water at xii. 30 p.m., November 6th. Treated in the same manner as the branches of *S. nigra* it was fixed in the apparatus at iii. 00 p.m., and supplied with supersaturated air during the rest of the afternoon, night, and following forenoon. On November 7th the rate of index-movement from xi. 00 a.m. to noon averaged 1·847 mm./min. At noon supersaturated nitrogen was supplied. Approximately the same rate was maintained till i. 12 p.m., then it gradually declined towards zero, becoming 0·012 mm./min. at ii. 40 p.m. At ii. 41 p.m. supersaturated air was turned on, and a quick recovery resulted (Fig. 5). The recovery, though very marked, was not permanent, and next day the rate scarcely touched 1·000 mm./min., and the depression caused by the introduction of supersaturated nitrogen was correspondingly inconspicuous (Fig. 6).

On November 15th an experiment was made with a shoot of *Clematis montana* grown in the open. The main axis of this shoot was 110 cm. long, and carried two side branches 50 cm. and 36 cm., respectively, in length. This whole carried 42 leaves. It was cut, treated, and fixed in the apparatus with the usual precautions, and then supplied with supersaturated air for 20 hours. Next day, November 15th, the rate of index-movement from xi. 04 a.m. to i. 04 p.m., while the supply of supersaturated air was still maintained, averaged 1·326 mm./min. At i. 04 supersaturated nitrogen was admitted into the bell, and for forty minutes the rate was practically unaltered (Fig. 7). Then it suffered a rapid diminution, reaching zero at ii. 20, and actually showed a very slight reversal from ii. 40 to ii. 50 p.m. Supersaturated air was then admitted, and was followed by a sharp rise in the rate of transpiration, and the movement of the index at iii. 18 had risen almost to its original value, viz., 1·250 mm./min. (Fig. 7).

At the conclusion of this experiment the bell forming the leaf-chamber was opened, and the branch was exposed to normal but somewhat restricted transpiration during the night and following morning. At ix. 30 a.m. on November 16th the leaves and walls of the chamber lined with blotting paper were sprayed with water, the closing plate was replaced on its soaked gasket of bibulous paper, and the usual supersaturated air-current started. From

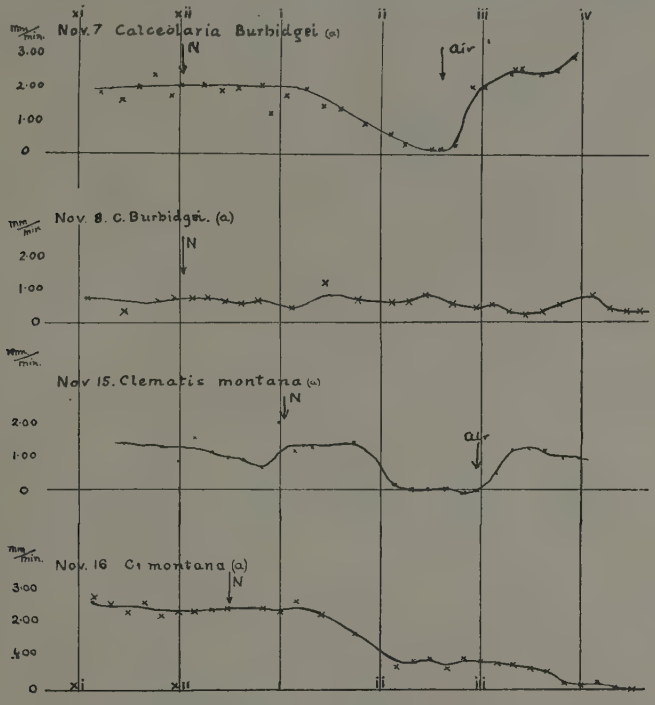

FIG. 5

FIG. 6

FIG. 7

FIG. 8

xi. 04 a.m. to xii. 34 p.m. the average index-rate was 2·450 mm./min. Supersaturated nitrogen was then admitted, and from xii. 34–i. 35 p.m. the average rate was still 2·412 mm./min.; from i. 35–ii. 05 p.m. it was 1·725 mm./min.; from ii. 05–iii. 45 it was 0·770 mm./min.; from iii. 45–iv. 15 p.m., 0·200, becoming zero at iv. 25 p.m. (Fig. 8). Then supersaturated air was admitted and continued through the night on into the next day when from xi. a.m. to xii. 20 p.m. the rate was 0·395. On the evening of November 18th, i.e. after the branch had been 4½ days in a supersaturated atmosphere, the rate of movement was still 0·100 mm./min.

Abutilon sp.? also gave material for these experiments. The species used is horticulturally known as "Boule de Neige." The leaves are large, having a blade about 100 sq. cm. It is easily manipulated, and stands up to the experimental exigencies extremely well. On November 22nd a shoot 44 cm. long, carrying 9 mature leaves, was introduced, after the usual preparatory

treatment, into the apparatus. It was supplied during the afternoon and night with a current of supersaturated air. Continued condensation on the

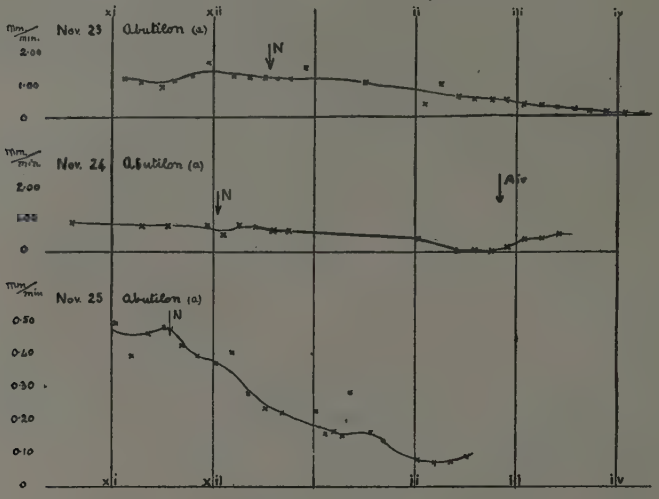

FIG. 9

FIG. 10

FIG. 11

leaves was apparent by the continuous dripping of water from the leaves. On the forenoon of November 23rd between x. 00 a.m. and xii. 36 p.m. (Fig. 9) the rate of movement of the potometer-index varied between 0·897 and 1·601 mm./min., the average being 1·158 mm./min. At xii. 36 p.m. supersaturated nitrogen was supplied, the rate continued over 1·000 mm./min. till i. 30 p.m. Then it slowly and regularly declined till it became zero at iv. 15 p.m. At iv. 20 p.m. supersaturated air was passed in, and continued on into the next morning. The rate of movement observed during the forenoon of November 24th was about 0·800 mm./min. (Fig. 10). Supersaturated nitrogen was again introduced a few minutes after xii o'clock noon, and the rate of movement slowly declined to zero about ii. 25 p.m. At ii. 50 p.m. the supply of supersaturated nitrogen was replaced by one of equally supersaturated air, and a gradual rise resulted. This supply was kept on through the night into the morning of the 25th, and the rate of movement found between xi and xii on that forenoon fluctuated between 0·490 and 0·395 mm./min. Supersaturated nitrogen was supplied at xi. 26 a.m. and the decline, shown with change of scale in Fig. 11, was observed. Here again the persistence of transpiration into a supersaturated atmosphere for several days is clearly demonstrated, and throughout the experiments lasting 4 days the leaves retained a perfectly fresh and healthy appearance, and showed no sign of falling or of discoloration.

Attention may be directed to certain points in the foregoing experiments :— Ample time (20 hours or longer) was given to the experimental branch, either

submerged in water or surrounded with a supersaturated atmosphere, to fill itself to repletion. Furthermore, it was cut from the parent plant under water which had shortly before been boiled, and was consequently almost free from dissolved air. After cutting it was supplied with changes of boiled water below. These precautions secured that the component cells of the branches were completely turgid, and the tracheae filled with continuous water-columns before the eosin was presented to the cut surfaces, or the movement of the potometer index was recorded. Hence neither the rise of eosin nor the upward movement of the index can be attributed to water-deficits in the stems. Again, in a large number of experiments, many of which are not described in this paper, the rise of the eosin was not limited to the tracheae of the stems and petioles, but extended out into the finest ramifications of the veins, showing that after the application of the eosin at the base, the whole tracheal system of the submerged branch had been emptied by subaqueous transpiration. In contrast to these precautions, in experiments quoted by critics (3) the time deemed sufficient to satisfy the possible water-deficit of the branch was 1–2 hours. The injection with eosin of the ultimate ramifications of the leaves transpiring subaqueously does not seem to have been observed by them.

Another feature of those experiments in which the branch was supplied with supersaturated gases was the length of time during which transpiration was observed. In several cases transpiration was observed into the fourth day after the branch had been injected with water for 20 hours or more.

The graphs show that a considerable time elapsed between the moment at which nitrogen was supplied and its effect on the rate of transpiration was observed—usually 60 minutes or more. Once the depression was initiated, however, complete inhibition usually followed fairly quickly. The irregularities observed in this course of events are probably to be attributed to the smallness of the supply compared with the volume of the leaf-chamber, and to the necessarily uncertain action of convection currents bringing the nitrogen into contact with the blades of the leaves.

The excellent condition maintained by the Abutilon shoot under the experimental treatment suggested that it would afford suitable material for studying the effects of anæsthesia on transpiration into saturated spaces.

In the first experiment in this direction it was attempted to apply chloroform to the transpiring branch by introducing 5 c.c. of chloroform into the bubbling flask E (Fig. 1), in the expectation that the air-stream would carry sufficient chloroform vapour with it into the leaf-chamber to effect the anæsthetisation of the transpiring cells. After 3 hours, however, this method of applying the chloroform produced no sensible effect on the rate of transpiration (Fig. 12).

On the next day, using the same branch, it was arranged that the supersaturated air-stream coming from the bubbling flask, by passing through a bottle (C, Fig. 1) containing a mass of cotton wool soaked in chloroform, should carry the vapour into the leaf-chamber. A striking result (Fig. 13) was obtained. The material, which on the day before had not responded to the previous treatment,

now exhibited a marked response. Since the last experiment it had been supplied with supersaturated air, and still exhibited a rate of index-movement of 1·439 mm./min. Chloroform vapour was introduced into the supply at xi. 22 a.m., and by xi. 40 a.m. the index had come to a standstill and five minutes later was moving in a downward direction. At xi. 51 a.m. the source of chloroform vapour was removed, and pure supersaturated air supplied. A

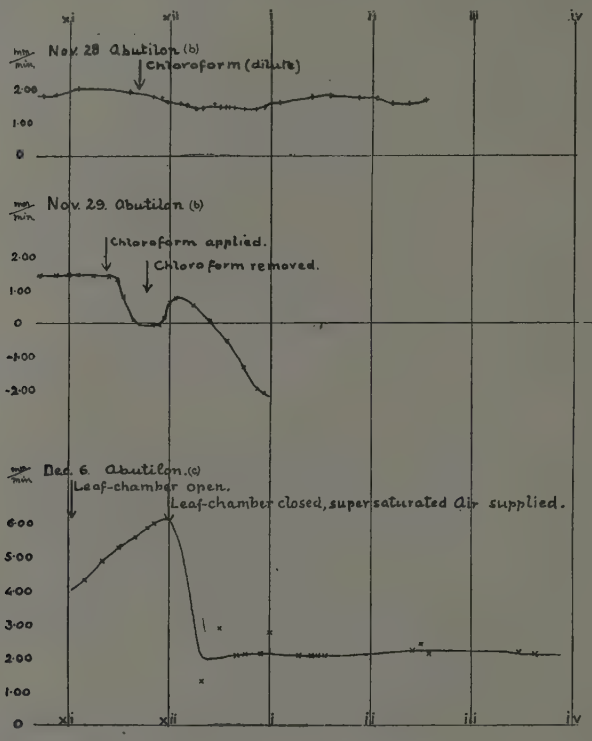

Fig. 12

Fig. 13

Fig. 14

quick recovery followed, and an upward movement of 0·770 mm./min. was recorded at xii. 05 p.m. This response to the air was only short-lived, and by xii. 25 p.m. upward movement was suspended, and downward movement began. The rate of downward movement continued to increase, and at i. 00 p.m. amounted to 2·020 mm./min. when the experiment was broken off.

Evidently the lethal action of the chloroform continued after the supply was removed, and had destroyed the transpirational mechanism of the cells, effective in saturated spaces, and now the plant was behaving as the physical system imagined at the outset.

Experiments were made also to compare the rate of this transpiration into supersaturated spaces (subaqueous transpiration) with normal transpiration under partially saturated conditions.

A shoot of Abutilon was set up with the usual technique, and supplied

during the following evening and night with a supersaturated air stream, while a piece of ribbed glass partially closed the bell glass acting as the leaf-chamber. At x.00 a.m. next morning this piece of glass was removed so that the bell glass communicated freely with the air of the laboratory, and a record was kept of the rate of movement of the potometer-index. At xi.00 a.m. (Fig. 14) the rate was 4·375 mm./min. It rose gradually to 6·020 at xii. 00. Then the bell was closed with the plane piece of glass with the soaked gasket of bibulous paper, while the supply of supersaturated air was kept up. At i. 17 p.m. the rate had fallen to 2·065. Here then the subaqueous transpiration amounted to one-third of the total transpiration into an atmosphere heavily charged with moisture.

A previous experiment made with a branch of *Ribes sanguineum* on July 25th examined the same question, and is recorded in Fig. 15. This time the apparatus was set up in a well-lighted and ventilated room. The air temperature was 19·4° C. The leaf-chamber was open to the room, and the rate of movement of the index varied from 5·000 to 7·600 cm./min. from x. 40 a.m. to xii. 10 p.m. At xii. 11 p.m. the leaf-chamber was filled with aerated water at 19·3° C. This reversed the current in the index-tube for the next 2 minutes, so that the rate was −0·700 cm./min. This momentary backward movement was probably entirely caused by the yield of the rubber bung closing

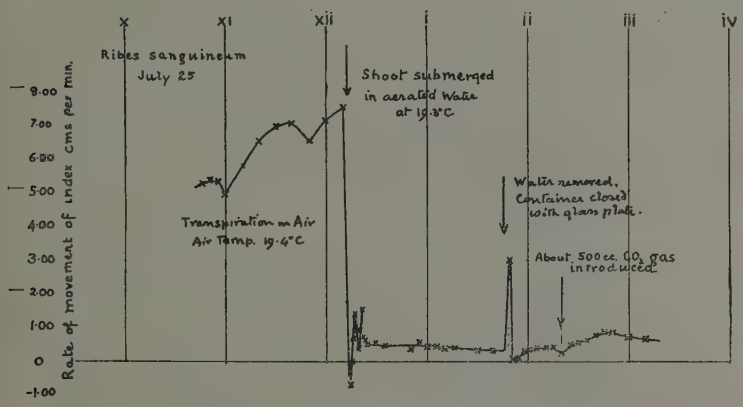

FIG. 15

the potometer to the head of water put into the leaf-chamber. The rate of movement was zero at xii. 15 and rose to 1·400 at xii. 16½ p.m. It ran down again to 0·350 cm./min. and rose to 1·550 at xii. 20½. These very fluctuating readings were possibly due to the readjustments of the connection to the new pressure conditions due to the filling of the leaf-chamber with water. At xii. 23 these adjustments were apparently completed, and the rate steadied at 0·500 cm./min. This was followed by a slow falling-off till 0·320 cm./min. was reached at i. 40 p.m. At i. 48 p.m. the water was siphoned out of the leaf-chamber and the latter was closed with the plane glass plate. A sudden rise in

the rate of index-movement followed by an equally sudden fall resulted at i. 50. Both these were due probably to the elastic reaction of the potometer-bung to the sudden reduction in head by the removal of the water from the leaf-chamber. Then the rate of index-movement rose to that obtaining when the branch was submerged and corresponds no doubt to the rate of subaqueous transpiration. A noticeable fall in this rate was observed after twenty minutes; but this fall was more than counteracted by passing into the chamber 500 c.c. of carbon dioxide gas saturated with water vapour. Possibly the oxygen set free by photosynthesis increased the respiratory activity of the transpiring cells, and was, in part at least, responsible for this change.

Summary.

Several examples of subaqueous transpiration are described. In these, leafy branches of various species, specially treated to secure that no water-deficiency obtained in their tissues, drew up eosin-solution in their stems for distances of 15–69 cm., while the leaves were completely submerged in water. In some cases not only were the tracheae in the stems injected with the solution of the dye, but even those in the very ultimate ramifications of the veins in the leaves also. Saturation of the surrounding water with dissolved air, and the evolution of oxygen in photosynthesis, appear to favour this subaqueous transpiration.

A potometer method for comparing the rates of transpiration in gases supersaturated with water is described, and it is shown experimentally that the replacement of air with nitrogen round the leaves depresses, and ultimately inhibits, the upward movement of water in the shoots. Renewal of air round the leaves may restore the activity. Further, anæsthetisation with chloroform markedly reduces, or stops the upward movement; and, at least temporary, recovery may be attained by bathing the leaves in air supersaturated with water. If the supply of supersaturated nitrogen or chloroform vapour is prolonged, reversal of the upward current may be produced, the shoot behaving like a dead system in which the water condensed upon the leaves flows downwards under the existing head.

In conclusion we would like to express our indebtedness for much helpful discussion during the writing of this paper to Dr. H. H. Poole.

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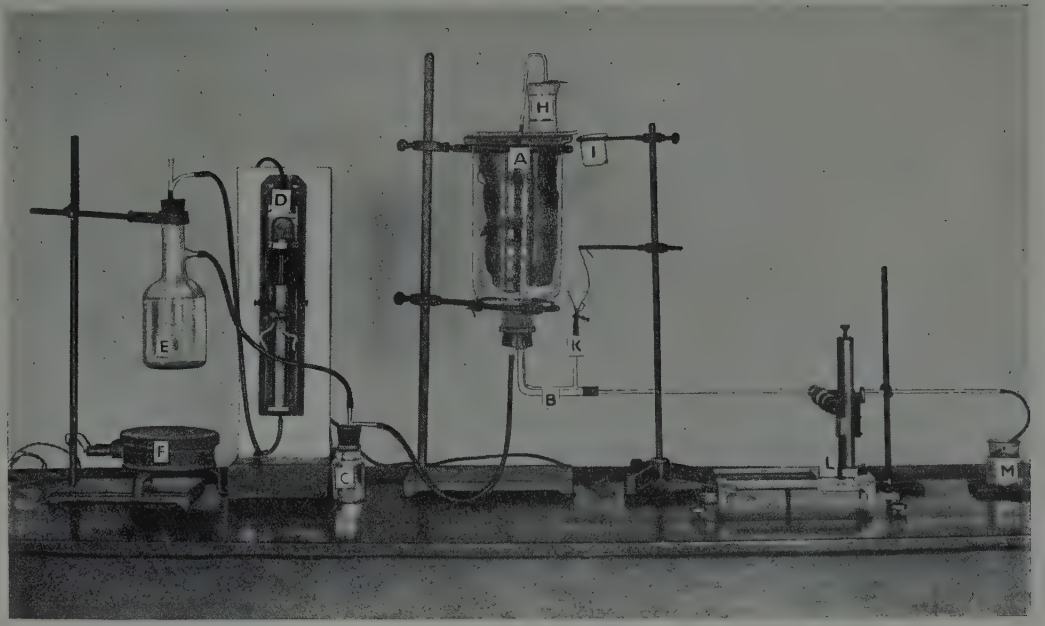

FIG. 1.

A, glass bell acting as leaf-chamber enclosing the experimental branch and the upper end of the potometer, **B**. **C**, a bottle containing cotton-wool saturated in chloroform, which may be inserted in the gas supply. **D**, electric air pump which drives the gas-stream through the bubbling-flask, **E**. **F**, electric hot plate which maintains the temperature of the bubbling-flask above that of the leaf-chamber. **H**, small beaker containing water trapping the exit of the gas-stream. **I**, small beaker forming capillary water-supply to the gasket between the glass-plate and the upper rim of the leaf-chamber. **K**, a side tubulure to facilitate the adjustment of the position of the index in the horizontal tube of the potometer. **L**, microscope on travelling stand to observe the positions of the index in the horizontal tube. **M**, water-supply for the potometer.

No. 21.

STABILISED DIAZO COMPLEXES WITH PIPERAZINE AND OTHER BASES.

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AND
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[Read February 27. Published separately March 15, 1940.]

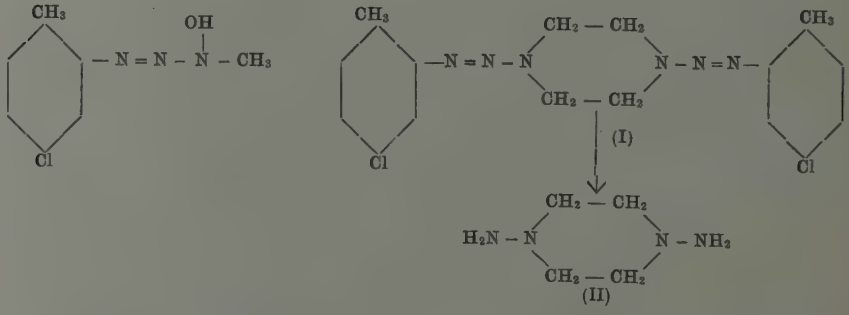
The marketing of stable diazo preparations is a well recognised problem in the dye industry. To enable the dyer to avoid the preparation of diazonium salts in the dye-house, several processes have been adopted for supplying the users with the diazonium compound in a readily accessible form. Although many processes are in existence for stabilising diazonium salts, the patent literature from year to year contains many specifications on this subject. These provide new processes or suggest improvements in the older methods. One of the first attempts at a solution of this problem was made by Schraube and Schmidt (Ber. 1894, **27**, 514). This was the subject of a patent by the Badische Anilin-und Soda Fabrik. (G.P. 78874). A dilute solution of p-nitrodiazobenzene chloride was quickly added to a moderately strong solution of sodium hydroxide at 50–60° C., a yellow solid was obtained which no longer combined with β-naphthol, and which was considered to be p-nitrophenyl nitrosamine. From the product, called "Nitrosamine Red in paste," on the addition of mineral acid, the diazonium salt is regenerated.

Another of the older processes is that of Meister, Lucius, and Brüning (G.P. 85387), where a dry stable powder is obtained by mixing the partially evaporated acid solution of the diazonium salt with anhydrous inorganic salts such as calcined alum or anhydrous sodium sulphate. The substance produced in this way from diazotised p-nitroaniline is marketed under the name of "Azophor Red P.N." Griess (Ann. 1866, **137**, 52) recognised the basic nature of diazonium salts, and prepared platinichlorides and aurichlorides. Later the combination of diazonium salts with several metallic and non-metallic chlorides was recorded. Some of these double salts have since been specified as stabilised diazonium salts. The double salt is first prepared, and is then collected, pressed, and dried by the addition of partially dehydrated inorganic salts. Another method of approach to this problem is due to Becker (G.P. 80139), who found that the addition of a solution of naphthalene sulphonic acids to diazonium salts gave diazonium naphthalene sulphonates of the type $C_6H_5N_2 . SO_3 . C_{10}H_7$. Those derived from naphthalene α-sulphonic acid, unlike the corresponding β-acid compounds, were sparingly soluble and thus easily

isolated. Other stable diazo preparations capable of being dried and ground are obtained by evaporation in vacuo in presence of neutral naphthalene di- or tri-sulphonates, fluorosulphonates, fluoborates, and p-chlorobenzene sulphonates. Among the interesting diazo preparations where the phenol required for coupling is included in the paste are the water-soluble mixtures containing 2–3 hydroxynaphthoic arylamides. These are introduced into the printing paste while in the alkaline condition, and on acidification and heating (e.g. with acetic acid) decompose to form diazonium salts, which immediately combine with the aryl amide to give the dyestuff. Stabilised diazo complexes have been formed by coupling diazonium salts with secondary amino acids, e.g. sarcosine $NHMe.CH_2.COOH$. These diazoamino compounds are known as rapidogens, and owe their water solubility, as sodium salts, to the presence of the carboxyl group. Diazo compounds react with aldehyde-bisulphite derivatives of ammonia or amines to give soluble stable diazo-amino compounds of the type $Ar.N:N.NR.CHR'.O.SO_2Na$, which, with mineral acids, regenerate the diazo salts.

In the present work the formation of new diazo complexes by the reaction of diazonium salts with substances containing an imino group has been studied. It was found possible to prepare from piperazine and β-methylhydroxylamine, in excellent yield, diazo-imino compounds, which on treatment with acid readily broke down again, yielding the diazonium salts.

The reaction with piperazine could conceivably give rise to products of two types: (*a*) one diazonium group reacting with the piperazine, or (*b*) two molecules of the diazonium salt coupling with the piperazine to give an N-N′-derivative of piperazine. That the second alternative is correct was proved by the reduction of this complex (I), and the isolation of N-N′-diamino piperazine (II).



This double coupling of the diazonium salt with piperazine is of special importance, as it increases the proportion of available diazonium salt in the complex.

The action of hydroxylamine, and that of β-methyl hydroxylamine on diazonium salts leads to an interesting comparison. It is well known that

phenyl azoimide, Ph.N$\begin{matrix}\diagup\!\!\diagdown\text{N} \\ \|\ \ \| \\ \diagdown\!\!\diagup\text{N}\end{matrix}$ is only formed in traces from diazobenzene and ammonia, but can be obtained readily from diazobenzene perbromide and ammonia. If hydroxylamine is used in the place of ammonia the arylazoimides can be obtained directly from the diazo salt. In the case of aryl or alkyl hydroxylamines (cf. Bamberger, Ber. 1896, **29**, 103) the compounds formed do not decompose to give phenyl azoimide, and are of the type $C_6H_3N:N.\overset{OH}{\underset{|}{N}}.R$. In the present work three technically important amines were chosen, 4-chloro-o-toluidine, 2-5-dichlor-aniline, and 5-chloro-o-toluidine. These were diazotised and added to solutions of piperazine and β-methyl hydroxylamine in sodium acetate solution. The complexes, which were formed in almost quantitative yield, were isolated, and their breakdown with acid investigated (see page 226). The regenerated diazonium salt was estimated as the azo-β-naphthol derivative.

EXPERIMENTAL.

(1) *Complexes with Piperazine.*

Di(3-chloro-6-methylbenzene-1-azo) piperazine.

19 g. of 4-chloro-o-toluidine were diazotised in the ordinary way and slowly added, with stirring, to a cooled solution of 23·2 g. of piperazine in excess of sodium acetate solution. The precipitate was washed with water, and on crystallisation from alcohol, in which solvent it is sparingly soluble, was obtained in long, faintly yellow, needles melting at 160·5°. Found N, 21·51%; $C_{18}H_{20}N_6Cl_2$ requires N, 21·48%.

Di(4-chloro-6-methylbenzene-1-azo) piperazine.

This compound, obtained in a similar manner, crystallised from alcohol in colourless prismatic needles melting at 184°. Found N, 21·56%; $C_{18}H_{20}N_6Cl_2$ requires N, 21·48%.

Di(2, 5-dichlorbenzene-1-azo) piperazine.

This compound is sparingly soluble in alcohol, from which it crystallises in long colourless needles melting at 146°. Found N, 19·28%; $C_{16}H_{14}N_6Cl_4$ requires N, 19·44%.

(2) *Complexes with β-methyl hydroxylamine.*

3-chloro-6-methylbenzene-1-azohydroxyamino methane.

49·1 g. (1 mol.) of 4-chloro-o-toluidine was diazotised and added to a cooled solution of 8·3 g. (1 mol.) of β-methyl hydroxylamine in sodium acetate solution. A copious ppt. of the complex was immediately formed. This was

collected and crystallised from alcohol, in which it dissolved readily on heating. It formed colourless prismatic plates melting at 76°. Found N, 21·12%; $C_8H_{10}N_3Cl\,O$ requires N, 21·05%.

4-chloro-6-methylbenzene-1-azohydroxyamino methane.

This compound is fairly soluble in hot alcohol, from which it crystallises in colourless prismatic needles melting at 84°. Found, N, 20·98%; $C_8H_{10}ON_3Cl$ requires N, 21·05%.

2-5-dichlorobenzene-1-azohydroxyamino methane.

This compound crystallises from alcohol, in which it is moderately soluble on heating, in colourless plates melting at 112°. Found N, 18·83%; $C_7H_7ON_3Cl_2$ requires N, 19·09%.

(3) *Evaluation of the Amount of Diazonium Salt recoverable from the Complexes when broken down by the Action of Mineral Acid.*

A weighed quantity of the complex was heated with 80 per cent. sulphuric acid to about 45°. In each case the complexes were found to dissolve readily with no noticeable evolution of nitrogen. The acid solutions were immediately cooled, and added to a solution of β-naphthol in sodium hydroxide. The filtered dyestuffs were washed, dried, and weighed. The percentage recovery of the diazonium salts is given in the following table:—

Diazonium Complex.	Percentage Recovery of Diazonium Salt.
4-Chloro-o-toluidine + piperazine	97 %
5-Chloro-o-toluidine + piperazine	74 %
4-Chloro-o-toluidine + methylhydroxylamine ...	74 %
5-Chloro-o-toluidine + methylhydroxylamine ...	55 %
2-5-Dichloraniline + methylhydroxylamine ...	97 %
2-5-Dichloraniline + piperazine	98 %

N-N'-Diamino piperazine.

Two g. of di(3-chloro-6-methylbenzene-1-azo) piperazine were dissolved in a mixture of alcohol and glacial acetic acid. Zinc dust was gradually added, and the whole heated on a water bath at 70° until reduction of the complex was complete. This was ascertained by withdrawing portion from time to time and testing for unchanged complex by heating with mineral acid, followed by addition of the acid solution to an alkaline solution of β-naphthol. When a negative test was obtained the zinc dust was filtered off, the filtrate made alkaline with caustic soda, and the diamino piperazine distilled over in steam, the distillation being continued until the distillate no longer reduced Fehling's

solution. The distillate, after the addition of hydrochloric acid, was brought to a small volume in a vacuum. The di-hydrochloride which separated was crystallised from alcoholic hydrochloric acid. On decomposing the di-hydrochloride with alkali the free base, diamino piperazine, was obtained, and on crystallisation from aqueous alcohol, melted at 100° (cf. Schmidt and Wickmann, Ber. **24**, 3245).

SUMMARY.

The combination of diazonium salts of technically important amines with substances containing an imino group has been studied. Stable diazo complexes of the type 2-5-dichlorobenzene-l-azo-hydroxyamino methane and di(4-chloro-6-methyl-benzene-l-azo) piperazine have been obtained from the reaction with β-methyl hydroxylamine and piperazine respectively. These complexes were readily broken down by acid, and the percentage recovery of the diazonium salts estimated. That the piperazine interacts with two molecules of the diazonium salt has been proved by reduction and isolation of N-N′-diamino piperazine.

The authors desire to thank Imperial Chemical Industries, Ltd. (Dyestuffs Group), for a grant, and also for gifts of chemicals.

No. 22.

THE REQUIREMENTS OF THE PIG FOR VITAMINS A AND D.

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(Plate 6.)

[Read January 23. Published separately April 1, 1940.]

Although a considerable amount of work has been done on the requirements of the growing pig for vitamins A and D, there is no general agreement as to the circumstances in which deficiencies are likely to occur. Thus Dunlop (1), at Cambridge, came to the conclusion that unless the Ca/P ratio lay outside certain limits, or the P content was below a certain minimum, the small amount of vitamin D contained in the cereal and protein meals of the basal ration was sufficient for the requirements of the pig between weaning and bacon weight. Similarly Foot *et al.* (2), at Reading, state that the value of vitamin D for pigs lies mainly in its therapeutic action when used in conjunction with diets of abnormal mineral composition. All these workers stress the importance of supplying vitamin A to the growing pig. Johnson and Palmer (3), however, at Minnesota, found that unless vitamin D was supplied pigs became rachitic a few months after weaning. Somewhat similar results were reported by Sheehy and Senior (4) from this department in 1930. It is evident from the literature that in the numerous experiments which have been carried out on vitamin requirements, the young pigs prior to weaning had been subjected to varied treatments in respect of vitamin supply. It was decided, therefore, to conduct an experiment in which they would be dependent mainly on their mother's milk for their requirements of vitamins A and D up to the time of weaning. The purpose of the experiment was to study the effect of deficiencies of the vitamins under these conditions, using rations composed of common feeding stuffs.

Experimental.

Three Large White sows were selected from the college herd in January, 1939, and placed on a meal ration of 40 parts of maize meal, 30 parts of pollard, 20 parts of bran, 10 parts of meat and bone meal. In addition they got green feeding and some whole milk. They had access to the open air at all times. Just prior to farrowing in April they were accommodated in houses

with a northern aspect. The doors were kept closed. Most of the light entering the houses passed through window glass. The sows were allowed out to the yards in the sunlight each day for as long as possible, but the young pigs were confined to the houses. No litter was provided, but portion of the floor was covered with wood. During the eight weeks of lactation the ration of the sows was as follows:—

20 parts Bran.
30 „ Pollard.
20 „ Flaked Maize.
12 „ Barley Meal.
10 „ Meat Meal.
5 „ Separated Milk Powder.
1 part Ground Limestone.
1 „ Steamed Bone Flour.
1 „ Common Salt.

It will be noticed that during pregnancy and lactation the sows were getting vitamins A and D. The 24 young pigs were weaned at about 8 weeks of age, and divided into 4 groups of 6 each, regard being paid to weight, sex, and litter in the usual manner. The composition of the basal rations fed is shown in Table 1.

TABLE 1.

Composition of Basal Rations.

	9/6/'39 to 30/6/'39	1/7/'39 to 22/7/'39	23/7/'39 to 12/10/'39
Bran	15	15	5
Pollard	35	35	40
Barley Meal	32	32	44
Meat Meal	6	9	8
Sep. Milk Powder	9	6	–
Steamed Bone Flour	1	1	1
Ground Limestone	1	1	1
Common Salt	1	1	1
	100	100	100
% Ca	1·57	1·77	1·69
% P	1·21	1·30	1·16
Ca/P ratio	1·30	1·36	1·45

Each group was fed according to appetite.

The different treatments which were given to the groups in respect of the supplies of vitamins A and D were as follows:—group 1—no vitamin supplement: group 2—vitamin A (concentrate): group 3—vitamin D (sunlight): group 4—vitamins A and D.

The vitamin A was supplied as a concentrate of high potency. It had a Blue Value of 2000. It was given to the pigs of groups 2 and 4 twice weekly, diluted with three times its volume of arachis oil, and mixed with the dry meals. A similar quantity of arachis oil was given to groups 1 and 3. The amount of the concentrate fed ranged from the equivalent of about 0·5 g. per pig daily at the commencement of the experiment to about 1·5 g. per pig daily towards the end. The great advantages of the use of this concentrate as the source of vitamin A were, firstly, that very large doses of vitamin A could be given with only a trifling increase in the energy-content of the basal ration; and, secondly, that the vitamin A was free from all traces of vitamin D. In order to make quite certain of this latter point the concentrate was fed to a group of 20 young chickens in a house lighted only through window-glass. In about 3 weeks practically all of the chickens had developed rickets, and they were cured by simply allowing direct sunlight to enter the house.

Groups 3 and 4 were supplied with vitamin D by placing them in houses facing west, and leaving the doors open during the daytime. As the experiment was conducted during the summer months it was considered that the sunlight would supply adequate amounts of vitamin D. In the case of groups 1 and 2, which were to be deprived of vitamin D, houses facing north were used, and the doors kept closed as much as possible.

Results.

In the second and third weeks of the experimental feeding there were 7 cases of mild scouring. It is worth noting that 6 of these occurred in groups which were not getting vitamin A. Three cases occurred in group 1 (neither vitamin), three in group 3 (no vitamin A), and one in group 4 (vitamins A and D). No great significance, however, can be attached to this distribution, as each group was in a separate house, and the cause of the scour could have been related to some factor connected with the particular house used.

The most significant occurrence in these experiments was the onset of rickets in groups 1 and 2. About eight weeks after the commencement of the different treatments, i.e., at 17 weeks of age, the pigs in groups 1 and 2 fell behind those in groups 3 and 4 in food consumption. Their skins became dirty, and their leg-joints became stiff. About 10 days later most of the pigs had deformed hind legs, and could move about only with the greatest difficulty, and apparently with great pain. Photographs of some typical cases are shown in Fig. 1 (Plate 6). A few days afterwards, on the 23rd August, there was only one pig in each group which appeared to be in normal health. On this date samples of blood from all of the pigs were taken, and the calcium-content determined. The Kramer-Tisdall method for serum calcium was used. As will be seen from Table 2, the level of calcium was definitely below normal for groups 1 and 2, except in the case of the two pigs which up to that time had not been affected. Since it was clear that the pigs were suffering from rickets,

and could make no further progress until cured, it was decided to give them access to sunlight. Three or four of the worst cases had to be lifted from the houses to the yard for the first 2 or 3 days. At the end of a week, during which time there was brilliant sunshine, all of them were able to walk, and three or four days later all signs of stiffness had disappeared. The hind limbs, of course, remained deformed in most cases. Their appetite rapidly improved, and they now put on weight at a higher rate than those in group 3, which had been getting vitamin D from the beginning. At the end of the experiment their blood calcium was again determined, and found to be at normal levels (Table 2). It is worthy of note that these pigs produced a very poor quality carcase at the bacon factory. A graphical record of the growth of the pigs is given in Fig. 3.

TABLE 2.

Calcium in Blood Serum (Mg. Ca per 100 ml.).

		28th July.	23rd August.†	12th October.
Group 1.	No. 32	11·2	10·9	12·3
	„ 30	10·6	8·4*	10·6
	„ 10	11·3	9·8*	12·2
	„ 15	9·5	7·5*	11·8
	„ 23	9·2	8·3*	13·2
	„ 20	7·3	—	—
Group 2.	No. 16	10·2	9·8*	11·2
	„ 31	11·8	12·3	12·5
	„ 9	9·6	8·2*	13·3
	„ 14	10·0	9·8*	12·0
	„ 24	9·5	7·8*	13·9
	„ 27	9·0	6·3*	10·9
Group 3.	No. 17	10·2	9·7	
	„ 22	10·4	11·0	
	„ 34	11·0	11·6	
	„ 12	10·8	11·0	
	„ 28	9·2	9·4	
	„ 25	10·1	11·7	
Group 4.	No. 33	11·0	10·8	
	„ 11	9·9	10·7	
	„ 18	10·3	12·9	
	„ 13	10·6	11·8	
	„ 26	11·2	12·4	
	„ 29	9·5	10·3	

* Visibly suffering from rickets.

† From this date Groups 1 and 2 were given access to sunlight.

Pig no. 20, in group 1, proved to be an exceptional case. From the date of weaning it made very little progress, and died after 9 weeks on experiment. About the 7th week its vision became defective, and it had difficulty in finding its way to the feeding trough. At the same time it developed the habit of carrying its head on one side, with almost continuous shaking of the head. During the following week it spent a lot of time walking around the pen in circles. As these symptoms corresponded with some of the symptoms of vitamin A deficiency described in the literature, heavy doses of vitamin A—200,000 Moore Blue Units—were administered each day for a week, but they failed to prevent death, possibly because the animal was in an advanced pathological condition. This pig was the only one in any group to show these symptoms. Fig. 2 (Plate 6) shows the appearance of the pig a few days before death. The constant shaking of the head made it a difficult subject to photograph.

Concerning the requirements of the growing pig for vitamin D, the inference to be drawn from the results of this experiment is that a deficiency may easily occur even where high levels of Ca and P are fed, and where the Ca/P ratio is about 1·4. Such a ratio would not be considered abnormal. The suggestion that the pig can be made independent of supplies of vitamin D, by proper balancing of the ration with respect to its calcium and phosphorus contents, apparently applies only to those cases where the vitamin has been stored during a previous period.

An examination of the growth curves of group 4 (Fig. 3) will show the beneficial effect of an adequate supply of vitamin A. These are the smoothest curves of all, and the growth rate is obviously faster. In 17 weeks they reached an average final live weight of 213 lb., as against 167 lb. for group 3, which did not get vitamin A. Both groups got vitamin D from the sunlight. Apart from this slower rate of growth there were no other obvious signs of deficiency or of ill-health in group 3. Although in Fig. 3 (for the sake of uniformity) the growth curves for group 3 are shown to the 12th October only, i.e., the date on which the pigs in group 4 were sent to the bacon factory, in fact the pigs in group 3 were kept on experiment until later dates. Three of them were sold on 14th November (31 weeks old), weighing 213, 214, and 231 lb., and the remaining three on 13th December, weighing 221, 233, and 233 lb. Even at these ages the pigs were quite normal and healthy in appearance. Apparently the small amounts of carotenoid pigments in the basal ration, while quite inadequate for a satisfactory growth rate, were nevertheless sufficient to prevent the development of the grosser symptoms of vitamin A deficiency—at least up to these weights. The meat meal did not contain vitamin A.

As a check on the adequacy of the amount of vitamin A administered, the livers of the pigs were assayed for vitamin A. The method of Davies (5) was used for the extraction. The vitamin was estimated by the Carr-Price Blue Test with antimony trichloride in chloroform. A Lovibond Tintometer was used for the colour comparison. The results are given in Table 3.

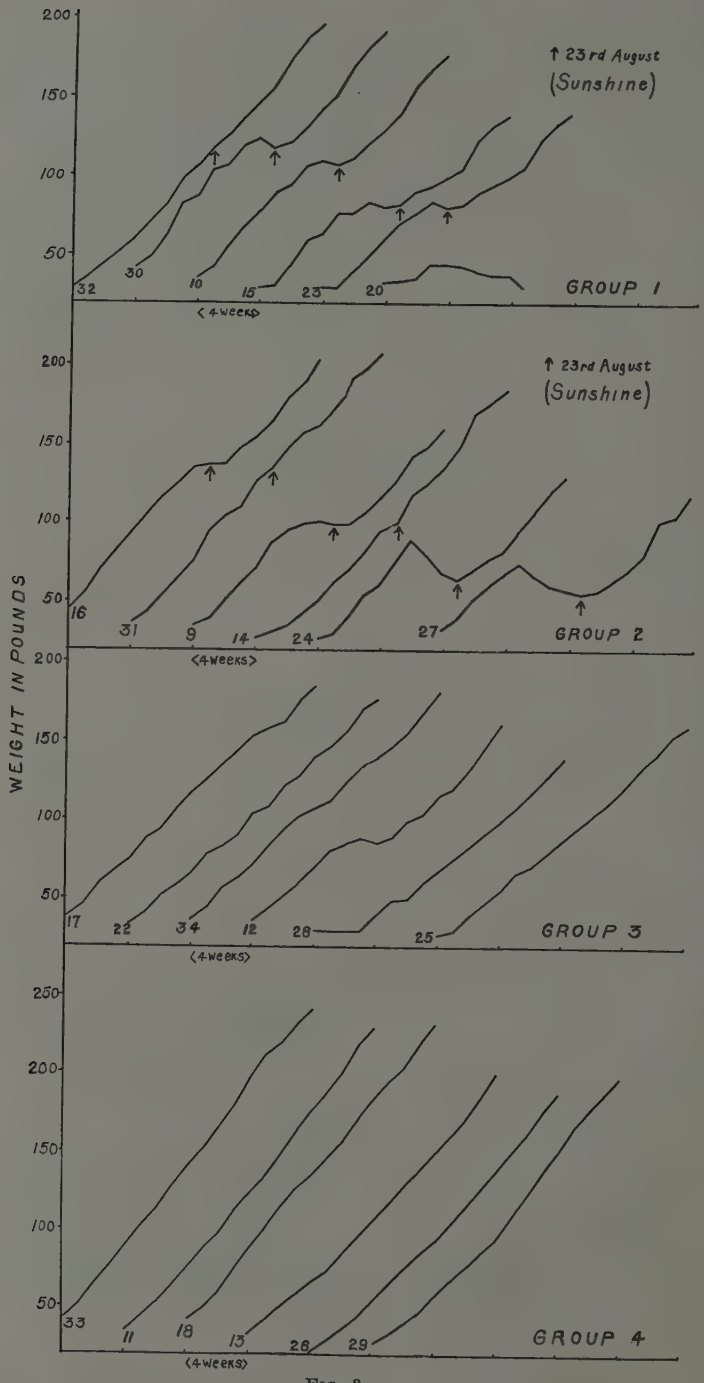
WEIGHT IN POUNDS
200
150
100
50
↑ 23rd August
(Sunshine)
32
30
10
15
23
20
GROUP 1
<4 weeks>
16
31
9
14
24
27
GROUP 2
17
22
34
12
28
25
GROUP 3
250
33
11
18
13
26
29
GROUP 4

FIG. 3.

TABLE 3.

Vitamin A in Livers.

	Pig No.	Moore Blue Units per gram of liver.		Pig No.	Moore Blue Units per gram of liver.
Group 1.	32	0	Group 3.	17	0
	30	0		22	0
	10	0		34	0
	15	0		12	0
	23	0		28	0
	20	–		25	0
Group 2.	16	375	Group 4.	33	500
	31	250		11	250
	9	400		18	550
	14	Trace		13	525
	24	125		26	375
	27	250		29	600

It will be seen that all the pigs in groups 2 and 4 were storing vitamin A in their livers. This is taken as indicating that adequate amounts were supplied in the ration. As was to be expected, there was no trace of vitamin A in the livers of group 1 or 3.

Table 4, giving the figures for live weight gain and economy of gain, shows in a striking manner the superiority of group 4 which got adequate amounts of vitamin A over group 3 which did not get a vitamin A supplement. Although the pigs of group 4 consumed 17 per cent. more food than those of group 3 they utilised it much more efficiently, as shown by the fact that the cost in food of a pound of live-weight increase was only 2·88 in group 4, as against 3·30 lb. in group 3.

TABLE 4.

Live-weight increase and economy of gain.

	Pig No.	Initial Weight (at 9 weeks).	Final Weight (at 26 weeks).	Gain in Weight.	
Group 3.	17	38	185	147	
	22	34	177	143	Average Increase per pig per day = 1·12 lb.
	34	35	182	147	
	12	37	160	123	
	28	29	140	111	Economy of gain = 3·30 lb.
	25	27	160	133	
	Mean	33	167	134	
Group 4.	33	43	241	198	
	11	35	223	188	Average Increase per pig per day = 1·50 lb.
	18	42	231	189	
	13	32	200	168	
	26	21	185	164	Economy of gain = 2·88 lb.
	29	27	197	170	
	Mean	33	213	180	

The difference between the mean weight increases of groups 3 and 4 is highly significant. The observed value of t (5·516) is much greater than that required (3·169) for significance at the 1 per cent. point for 10 degrees of freedom (6).

Summary.

Growing pigs require vitamin D even on a high calcium and phosphorus intake with a Ca/P ratio of 1·4. In its absence a gross pathological condition occurs, and the pig is incapable of making further progress until vitamin D is supplied.

The beneficial effects of adequate amounts of vitamin A are confirmed, and figures are given showing its remarkable effect on growth rate and on economy of gain.

Acknowledgments.

I wish to express my gratitude to Dr. E. J. Sheehy for direction in the planning of this experiment, and for his helpful criticism at all stages of it.

I am indebted to Messrs. Lever Bros. of Liverpool, from whom supplies of the vitamin A concentrate were obtained, and to Messrs. Kehoe & Co., Bacon Curers of Dublin, who gave facilities for the recovery of the livers.

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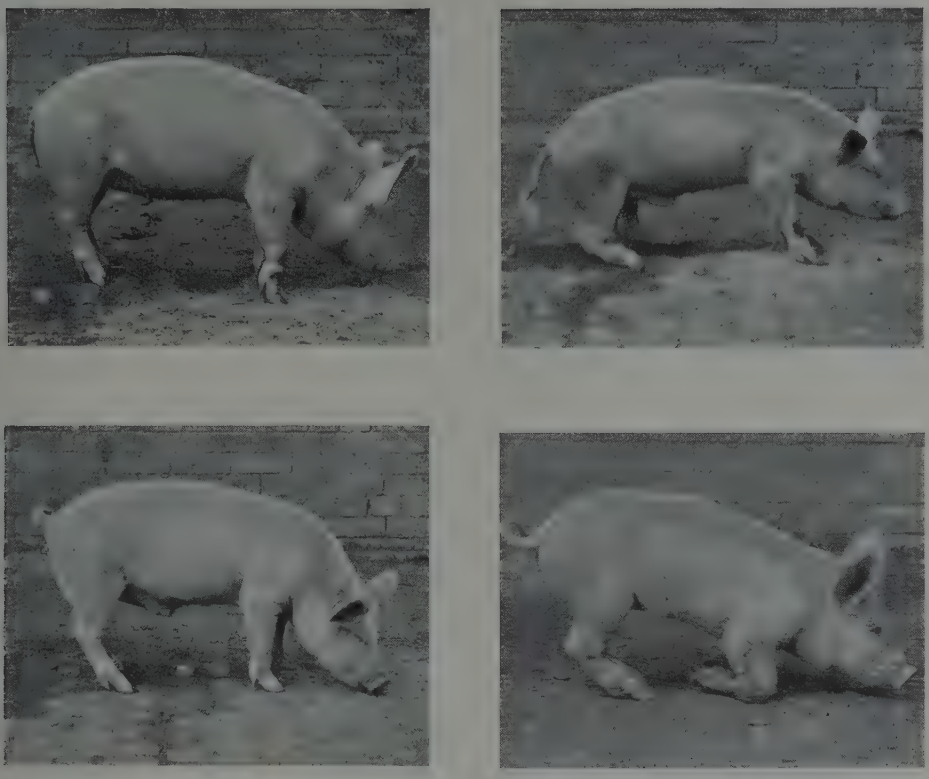

FIG. 1.—Rachitic pigs from Group 1 (upper pair) and Group 2 (lower pair).

FIG. 2.—Pig 20, Group 1 (see p. 233).

No. 23.

THE CHEMICAL CONSTITUENTS OF THE LICHEN *PARMELIA LATISSIMA* FÉE.

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AND
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[Read March 19. Published April 8, 1940.]

The lichen *Parmelia latissima* Fée is not found in Ireland, and has not hitherto been investigated. The sample which we have examined was obtained for us through the courtesy of the Director of the Royal Botanic Gardens, Kew, from Mr. C. C. Gray, of Changana Tea Estate, Kenya Colony, who has used the lichen as part of the compost in the planting out of orchids. Mr. Gray states that he has got very gratifying results when this lichen is used.

From the lichen we have isolated four main products, lecanoric acid, a mixture of atranorin and chloratranorin, salazic acid, and d-arabitol; the first of these is the predominant substance present. The occurrence of chloratranorin in this corticole lichen is of interest, and adds one more to the number of lichens which have been shown to contain chlorinated products.

Experimental.

Ether extract of lichen.

250 g. of the lichen was extracted at room temperature with three lots of ether each 500 c.c. The combined extracts yielded 18 g of a grey solid.

9 g. of the solid, on boiling with 75 c.c. benzene, gave 7·2 g. of an insoluble product. The benzene solution, on evaporation, gave a green resin containing some crystalline material; this was washed with 50 c.c. warm ligroin, and the residue crystallised from a mixture of equal parts of chloroform and alcohol. 0·4 g. of a mixture of atranorin and chloratranorin, m.p. 194° C., was thus obtained. The material was insoluble in sodium bicarbonate, soluble in sodium hydroxide; it gave a port-wine colour with ferric chloride, and no reaction with bleaching powder.

3·796 mg. gave 8·350 mg. CO_2; 1·720 mg. H_2O.
4·971 mg. gave 0·530 mg. AgCl.
Found C = 59·99; H = 5·07; Cl = 2·64.
70% $C_{19}H_{18}O_8$ and 30% $C_{19}H_{17}O_8Cl$ requires C = 59·41; H = 4·61; Cl = 2·64.

The portion of the ether extract insoluble in benzene was warmed with 80 c.c. chloroform, and filtered. On evaporation of the filtrate and crystallisation of

the residue from a mixture of alcohol and chloroform a further 0·2 g. atranorin was obtained. The material insoluble in chloroform was crystallised, first from 90 per cent. acetic acid, and then from aqueous acetone, yielding a substance m.p. 176° C.

4·390 mg. lost in vac. at 100° C. 0·230 mg. H_2O.
Found C = 60·38; H = 4·54; H_2O = 5·24.
$C_{16}H_{14}O_7$ requires C = 60·38; H = 4·40. $C_{16}H_{14}O_7 . H_2O$ requires H_2O = 5·36.

The substance has all the properties of lecanoric acid; it is sparingly soluble in chloroform, acetic acid, and benzene, in the cold, and is easily soluble in cold alcohol, acetone, and in warm acetic acid. It is soluble in sodium bicarbonate, and a solution in alcohol gives a purple colour with ferric chloride, and a blood-red colour with bleaching powder; the substance dissolves in sulphuric acid to a colourless solution.

The acetic acid mother liquors were evaporated under reduced pressure, and on extraction with ether gave a product which on crystallisation from a mixture of benzene and ligroin melted at 107° C., and on exposure to the air for some time melted at 57° C. The substance was orcin, and showed no depression in melting point when mixed with the latter. This orcin had obviously been produced from the lecanoric acid by hydrolysis.

0·1 g. of the lecanoric acid was dissolved in 5 c.c. acetone, and methylated with an ethereal solution of diazomethane (from 4 c.c. nitroso-methyl urethane). On evaporation of the solvent and crystallisation of the residue from 10 c.c. methyl alcohol the tetramethyl derivative of lecanoric acid was obtained, m.p. 145–146° C.

3·850 mg. gave 9·050 mg. CO_2; 2·040 mg. H_2O.
4·572 mg. gave 10·790 mg. AgI.
Found C = 64·11; H = 5·93; OCH_3 = 31·1.
$C_{16}H_{10}O_3 . (OCH_3)_4$ requires C = 64·17; H = 5·88; OCH_3 = 32·9.

The substance is insoluble in caustic soda; a solution in alcohol gives no colour reaction with ferric chloride or bleaching powder.

2 g. of the lecanoric acid was dissolved in 40 c.c. of 5 per cent. methyl alcoholic potash, and maintained at 40° C. in a hydrogen atmosphere for two hours. The whole was then cooled, 120 c.c. water added, and saturated with carbon dioxide. It was then extracted with ether, and the material recovered from the ether solution crystallised from benzene. A product was thus obtained, m.p. 140° C.; it was methyl orsellinate, and showed no depression in melting point when mixed with a sample of the latter.

4·086 mg. gave 8·920 mg. CO_2; 2·000 mg. H_2O.
3·920 mg. gave 5·230 mg. AgI.
Found C = 59·54; H = 5·44; OCH_3 = 17·6.
$C_8H_7O_3 . OCH_3$ requires C = 59·34; H = 5·49; OCH_3 = 17·05.

The aqueous bicarbonate layer was acidified, and the precipitate formed collected and crystallised from water. The substance had m.p. 177–178° C., and was orsellinic acid.

3·965 mg. lost in vac. at 100° C. 0·392 mg. H_2O.
3·573 mg. gave 7·490 mg. CO_2; 1·580 mg. H_2O.
Found C = 57·17; H = 4·97; H_2O = 9·9.
$C_8H_8O_4$ requires C = 57·14; H = 4·77. $C_8H_8O_4 . H_2O$ requires H_2O = 9·6.

Acetone extract of lichen.

After extraction with ether the lichen was dried and extracted with boiling acetone. On removal of the solvent from the extract a brown product was obtained weighing 5 g. This was boiled with 70 c.c. benzene, and the portion insoluble treated with 40 c.c. boiling methyl alcohol. The fraction insoluble in these solvents was crystallised from 80 per cent. acetone, when a substance decomposing at 245° C. was obtained; it was salazic acid.

3·490 mg. gave 6·810 mg. CO_2; 1·150 mg. H_2O.
Found C = 53·22; H = 3·69. $C_{18}H_{12}O_{10} . H_2O$ requires C = 53·2; H = 3·45.

With concentrated sulphuric acid the material gave a yellow solution which became orange on standing; with caustic soda a brown solution, and with ferric chloride a port-wine colour.

50 mg. was acetylated with 3 c.c. acetic anhydride and a couple of drops of sulphuric acid; after standing for two hours the solution was poured into water, and the precipitate crystallised from acetone. The resulting product had m.p. 178° C., and showed no depression in melting point when mixed with a sample of hexacetyl salazic acid.

Alcohol extract of lichen.

After extraction with acetone the lichen was dried and extracted with boiling ethyl alcohol. After removal of the solvent from the extract the residue was treated with 400 c.c. warm water, the insoluble portion discarded, and the aqueous solution evaporated to dryness. The residual gum was extracted with 20 c.c. alcohol, and the solution cleared with norite; to the solution was then added four volumes of dry ether. On standing large colourless crystals separated, which were recrystallised from a mixture of alcohol and dry ether. These crystals had m.p. 101° C., and showed no depression in melting point on mixing with d-arabitol.

0·1 g. was heated with 0·1 g. fused sodium acetate and 0·5 c.c. acetic anhydride for 30 min., and the product obtained on pouring into water, crystallised from ligroin. The acetate thus obtained had m.p. 74° C., not depressed on admixture with d-arabitol acetate.

No. 24.

NEW OBSERVATIONS ON THE LIFE-HISTORY OF *CALLITRIS*.

By W. J. LOOBY and J. DOYLE
(University College, Dublin).

[Read March 19. Published separately May 7, 1940.]

Our knowledge of the life-history of *Callitris* is at present limited to the facts presented thirty years ago by Saxton (1910). This paper, while establishing the essential importance of the developmental stages in the genus, was incomplete. Recently, however, a specimen of *C. rhomboidea*, growing under glass in the Botanic Gardens, Glasnevin, Dublin, was noticed to develop a reasonable number of healthy cones; and collections from it during the last few years have supplied data filling many of the gaps in Saxton's account. The paper here presented does not give a complete connected account in itself but must be considered in conjunction with Saxton's, which it supplements and in places corrects.

At an early stage in the collection, when it was clear that the specimen was fertile and would ultimately yield results, correspondence took place with Miss Alison Baird of the University of Western Australia who was known to be interested in the species native to that area. It was agreed, however, that co-operation across such a distance would be difficult, and that it would ultimately be more convenient to publish separately whatever results might become available. An interesting comparison might thus be made between western species of *Callitris* under indigenous conditions and an eastern species grown under glass in a north temperate region.

Description of Results.

The number of cones available for investigation being relatively few collections were limited to the more critical phases.

The gametophytes.—The gynospore mother cell is well seen about mid-May. In *Callitris rhomboidea*, there may be, as Saxton has already described for the species investigated by him, up to three potential mother cells. But, as shown in Fig. 1 A (cf. Saxton's figure, Pl. 45, 5), only one develops as a very distinct and typical functioning mother cell. The others, if present at all, may be distinguishable from the ordinary cells of the nucellus mainly by their larger nuclei, but only for a short period. In one case, however, a second one much more distinct than usual was found in an ovule at a later stage when the functioning gynospore itself had begun to expand. In Fig. 1 A a second non-functioning mother cell is drawn overlapping the functioning one a little at the base. At this stage no distinct tapetal zone is developed.

Tetrad formation was not followed in detail. The few stages available showed no unusual feature to justify a waste of ovules, a single tetrad being typical. Occasionally in older ovules more than one prothallus was met with, in one case three. This phenomenon was also described by Saxton, but it is reasonably certain that it is due to the germination of more than one of the spores of a single tetrad, and has little value for phylogenetic relationships, as it occurs frequently but sporadically throughout the conifers.

The functioning spore, already vacuolate while still uninucleate, develops into the usual young gametophyte with parietal protoplasm and embedded free nuclei. Two features of interest are shown at this stage. A small apical zone shows a greater condensation of protoplasm with a number of nuclei scattered through it. Further, at this stage in conifers, simultaneous division of all the parietal nuclei is usually described as taking place. In *C. rhomboidea*, however, divisions follow in a series of waves from the top downwards. In Fig. 1 B the general relations of prothallus and nucellus are indicated, and, to the right of the figure, the stages are drawn in which the nuclei were found in this particular ovule—at the top, the middle and the bottom respectively. In any one such section a whole series can be seen from early prophase to metaphase. The nuclei of the special apical zone keep step with those in the neighbouring upper parietal area. The gametophyte already occupies most of the nucellus, there being no obvious development of the "pavement tissue" as described by Saxton for *C. verrucosa.*

In the meantime the pollen tube starts its growth—small tubes at the top of the nucellus being found in the mid-May collections. These tubes are binucleate, but details up to the formation of the body cell were not followed. Growth and development are extremely slow during the months following pollination. Reference to the dates under the published figures shows that the gametophyte may still be in the vacuolate stage in early December. Activity is marked during this month, and early in January the young embryos are to be found in any ovule which is fertile. Development is fairly uniform in the ovules of any one cone, but there are usually considerable differences in the stages shown in different cones.

The young body cell is ovoid in form and a little corrugated in outline when seen in section (Fig. 1 D). The mature body cell, which was not described by Saxton, is shown in Fig. 1 E, with the two sterile nuclei preceding it down the tube. The hour-glass shape is quite distinctive, only a thin zone of protoplasm covering the nucleus at the waist. Cut transversely, it shows at both ends, through several sections, as a uniform circular mass of finely granular protoplasm (Fig. 3 A), an appearance contrasting markedly with the median transverse section (Fig. 1 G) in which the large nucleus surrounded by a thin skin of cytoplasm is seen.

The division of the body cell shows a long narrow spindle. The chromosomes, in telophase at least, are also much longer than those in any other gametophytic divisions (Fig. 1 F). Segmenting across the narrow zone the two young male

cells have the peculiar shape to be expected. They separate a little, and may become completely rounded off or may still show a slight flattening along the plane of division (Fig. 3 D). The nucleus, at first small, enlarges, and the cytoplasm becomes rapidly denser, due partly to the appearance of a large

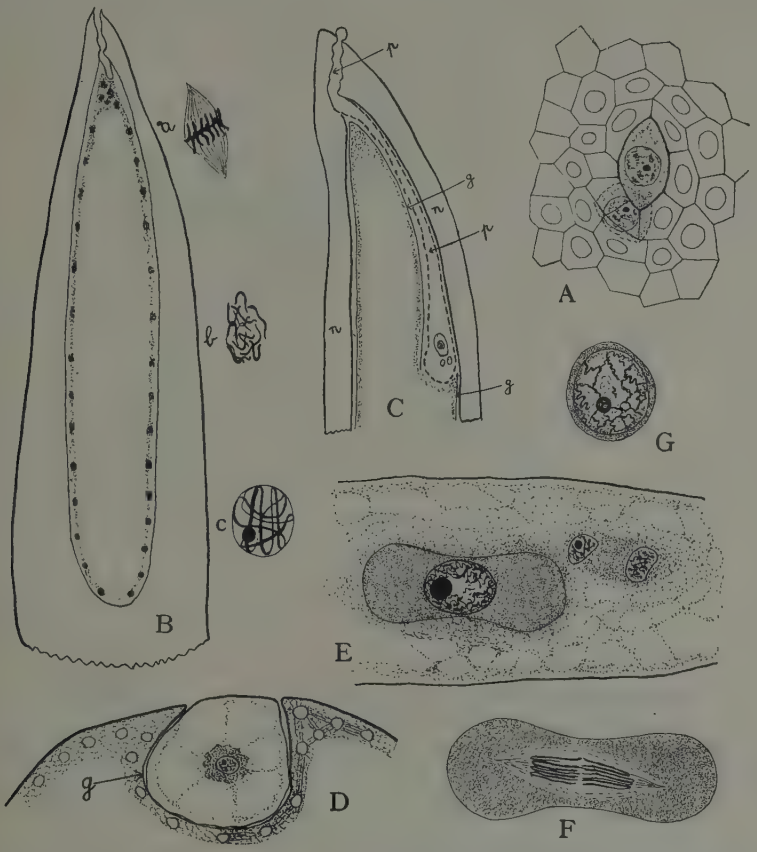

Fig. 1.—*Callitris rhomboidea.* **A.** Gynospore mother cell in nucellus with a second non-functioning one below. × 500. May 18. **B.** L.s. ovule to show relations of young vacuolate gametophyte (× 30); to the right the state of nuclei at the top *a*, middle *b*, and bottom *c*. (× 500). **C, D.** L.s. (× 60) and t.s. ovule (× 250) to show relations of pollen tube *p*, and spore membrane *g*. Dec. 6. **E.** L.s. mature body cell in the tube with the sterile nuclei. × 375. Dec. 3. **F.** Division of the body cell. × 500. Dec. 20. **G.** T.s. body cell through the waist. × 500. Dec. 3.

number of small starch grains. The mature male cells were described by Saxton, but not the body cell or their development. His figure (Pl. 45, 16) shows probably the young male cells just subsequent to the segmentation across the waist and before the definite appearance of the starch grains.

It is known from Saxton's paper that, in *Callitris,* the pollen tube grows along the side of the prothallus which develops lateral archegonia, but he does not clarify the relations of the pollen tube and the female gametophyte. One of his figures (Pl. 45, 12) strongly suggests that the pollen tube grows into the prothallial tissue, and that the archegonia are deep-seated. Deep-seated archegonia and an embedded pollen tube are, however, described by Saxton for *Actinostrobus* (1913, Pl. 25, 10), a genus which he correctly closely relates to *Callitris.* In these new collections a fairly wide range of stages was available in the development of the two gametophytes. The pollen tube advances while the prothallus is still vacuolate, and reaches approximately its final position (between $\frac{1}{3}$ and $\frac{1}{2}$ way down) before cell formation begins. Figs. 1 C, D show that it lies quite definitely outside the gynospore membrane. Fig. 1 D, a transverse section at the level of the body cell, shows that the tube (its course being from vertical to strongly oblique) comes to lie in a groove along the prothallus, the groove being lined by the gynospore membrane. Later on this groove may deepen, and the prothallus may extend round outside the pollen tube so that the two edges of the groove come into contact or even fuse (Fig. 2 A). When the prothallus is cellular a thin belt of tissue may be seen outside the pollen tube derived from this encircling growth, but the complete encirclement is not essential, since pollen tubes quite free on the outside may be seen in much older ovules. Fig. 1 C shows, very diagrammatically, a vertical section median to the groove in the case of a tube pursuing an even vertical course. Such a regular course is not common, but, when met with, shows the relations of the structures quite clearly in this plane also. At the extreme tip of the pollen tube, as this figure also shows, the prothallus may be a little invaginated; it is, however, never pierced. In this neighbourhood at this stage, and about the whole body cell zone later, the gynospore membrane may become so thin as to be undiscernible, the pollen tube membrane thinning markedly also. But the development shows quite clearly that the surface of the prothallus in contact with the tube is, from the beginning, the morphological outer surface. It would be interesting to follow the development in *Actinostrobus* from this point of view. It seems likely that there also the apparent embedding of the tube is a secondary phenomenon due to encirclement.

The early cellular development in the prothallus shows features of interest. The waves of successive division in the parietal nuclei come to an end and, immediately before the initiation of the alveolar stage, there follows a final simultaneous division, apparently really simultaneous through the whole extent of the female gametophyte. This division is at right angles to the surface, giving rise to the appearance somewhat diagrammatically shown in Fig. 2 A. It will be noticed, that, in relation to the encirclement of the pollen tube, a slight massing of nuclei has taken place (see also Fig. 1 D) lateral to the tube; but here also the plane of each mitosis is at right angles to the surface. A membrane is formed in each spindle periclinal to the outer surface, and from this division an outer layer of small cells is eventually formed. Fig. 2 B shows

a small zone in greater detail. Saxton mentions in *Actinostrobus* (1913) that alveoli are organized "each of which, before reaching very far towards the centre of the sac, cuts off a small cell at its base." The exact stage of the division is not figured or otherwise established, the figure drawn for *Actinostrobus* (Pl. 25, 9) showing alveoli already well advanced towards the centre. This phenomenon was not described by Saxton for *Callitris* (1910), and it is again suggested that, if stages were available for *Actinostrobus*, the origin of the layer

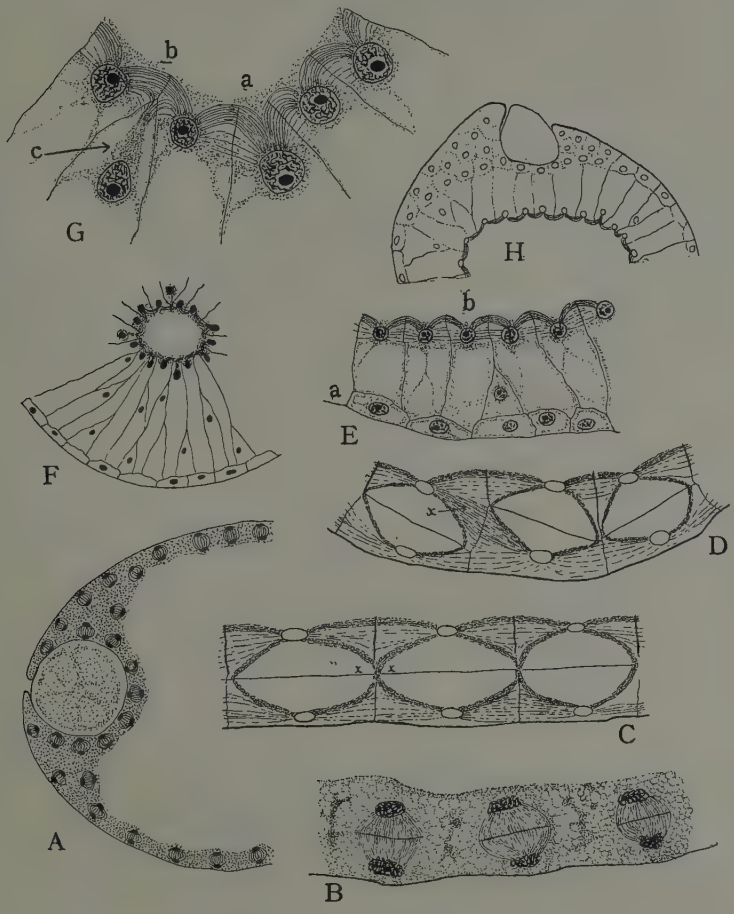

Fig. 2.—*Callitris rhomboidea.* **A.** T.s. of part of gametophyte to show nature of the last anticlinal simultaneous division (somewhat diagrammatic). × 180. Jan. 5. **B.** Small zone of A at a higher magnification. × 750. **C, D.** Diagrams to indicate probable origin of membranes round the outer nuclei and the initiation of the alveoli. **E.** Early stage in alveoli showing small cells *a* externally and, internally, the belt of nuclei *b* with interconnecting spindles. × 375. Dec. 21. **F.** Diagram to show nature of alveolar closure. × 120. Dec. 3. **G.** Inner nuclear zone of F. × 750. **H.** Alveolar development opposite pollen tube. × 120. Jan. 5.

of small outer cells in this genus would be very similar to the process here seen in *Callitris*. It has been described in no other conifer.

The exact formation and origin of the membranes surrounding these outer small cells present problems of which the solution is still a little uncertain. Before the initiation of alveolar formation in most conifers spindle connections exist between the parietal nuclei, appearing when fixed like delicate radiating fibres. They are quite distinct in *Callitris* also before this final anticlinal division, become less so during it (they are not drawn in Fig. 2 B), but become distinct again shortly after the stage shown in this figure. On them is laid down the outer anticlinal membranes. The spindle of each pair of dividing nuclei widens considerably, making contact with neighbouring spindles. The result may be very regular and symmetrical as in Fig. 2 C. Spindles also become established between the outer nuclei, and upon these the membranes of the alveoli proper are laid down. The membranes become connected by the gradual expansion and interconnecting of the spindles. Thus the two true mitotic spindles at x and x join and, splitting, form two periclinal spindles. The outer becomes associated with that of the outer nuclei, the whole of this spindle zone gradually fading as the formation of the anticlinal primary wall is complete. The inner one becomes joined with, if indeed it does not actually form, the innermost spindle connecting the inner nuclei, the spindle on which the alveolar membrane proper is deposited. On the other hand development may be less regular. The pairs of nuclei, resulting from the last simultaneous divisions, may become staggered, and the spindles between them, as well as the membrane forming on them, may lie obliquely, at times strongly so. In this case additional spindles may develop between the inner and outer nuclei of neighbouring pairs, such as the spindle x in Fig. 2 D. The resulting membrane formation may thus resemble that indicated in this figure. In zones where greater staggering of the nuclear pairs takes place still greater irregularity may be seen in the position of the membranes which enclose the outer nuclei. In all cases there is finally organised, lining the vacuole, a thin zone of protoplasm mainly composed of the single layer of nuclei interconnected by the spindles upon which membranes are deposited. The young primary alveoli are thus initiated.

The primary cell formation in the prothallus of conifers by the ingrowth of alveoli was first properly described by Sokolowa (1890). In many papers since published by various authors drawings are given of alveoli in a number of conifers. Most of them, however, give a very imperfect idea of the nature of alveolar formation and growth, and none of them indicate what seemed to Sokolowa the most important feature of her work, and what would still seem to be the most interesting aspect of it. Nor is this aspect referred to by Chamberlain (1935), or Schnarf (1933). As *Callitris rhomboidea* showed the point more clearly than any other conifer so far examined, brief reference may be made to it. The inward growth of the alveoli is controlled, as shown in Fig. 2 E, by the nuclei, which are connected by spindles. The spindles are denser on the inner side where they are strongly convex inwards, and lighter

opposite the nuclei. Each nucleus is connected to five or six of its neighbours, the contiguous spindles being practically in contact along their edges. On these spindles the primary membranes of the tubular alveoli are laid down. As these grow inwards the circumference of this nuclear zone must become continually shorter, and the ingrowing tubes must adapt themselves to this. This is done by the lagging behind of some of the nuclei, and the closure of the alveoli associated with them. This lagging and closure may begin quite early, and is continued throughout the inward growth. Fig. 2 F shows this diagrammatically, and shows also the first stage of the lagging of some of the nuclei in the zone surrounding the decreasing vacuole. Fig. 2 G shows how the closure is effected. As a nucleus falls behind, the spindles connecting it with the neighbouring nuclei dip down with it, the middle of the spindles approaching each other, as at *a*. As they do so the membranes developed on the spindles also approach and finally meet. The approaching spindles coalesce and soon join together nuclei previously separated by the closing alveolus, and, on this new spindle, as at *b*, the membrane continues inwards from the point of closure. The coalesced spindle zone of protoplasm, which had dipped down with the lagging nucleus, gradually reverts to the ordinary protoplasm type, and is visible for some time as a denser column of protoplasm *c* projecting up to the point of alveolar closure. Ultimately the nucleus passes back a little into the alveolus, and is ready to initiate the divisions which form the true cellular tissue later. These divisions do not occur until the inward growth of the primary alveoli has closed the prothallial vacuole. How the final closure is done was not properly determined. Comparatively few spindles are functioning by then, and cross-connecting spindles seem to develop between the nuclei approaching one another in the centre. Upon these spindles the final membranes meet to effect the closure.

The details of this process, which was clearly shown by Sokolowa fifty years ago, still require proper examination with careful technique. Although in the drawings here presented definite membranes are shown outlining the alveoli the nature of these membranes, whether protoplasmic or pectic, is far from clear; nor has the relation between these primary membranes and the walls subsequently laid down in the course of later cell division yet been elucidated. The varying and, at times, inconsistent accounts in the literature of alveolar formation in the different conifers seem, from many of the published drawings, to be due, partly at least, to a lack of appreciation of these central features in Sokolowa's work. Lawson's account in *Microcachrys* (1923), to mention but one case, can best be explained as a confusion between a true wall and a delicate spindle belt connecting the nuclei. The whole question, however, of primary alveolar development in the conifers needs separate discussion.

In *Callitris*, further, as shown in Fig. 2 H, the development opposite the pollen tube of alveoli based on an outer small cell (or membrane-covered protoplast) is quite similar to that at any other part. Some of these function as archegonial initials. The details of early archegonial formation were,

however, not followed. It could not be definitely ascertained, for example, if the small peripheral cells function as mother cells for the neck, though this is probable. As Saxton (1910) only records the mature archegonium, some reference to younger stages is desirable. The young archegonia are typical of this class of conifer, as shown by Fig. 3 A. The neck is composed of one tier of two or three cells (Fig. 3 B), although occasional slight irregularities may occur, as shown by the staggering of the neck cells on the left in Fig. 3 A.

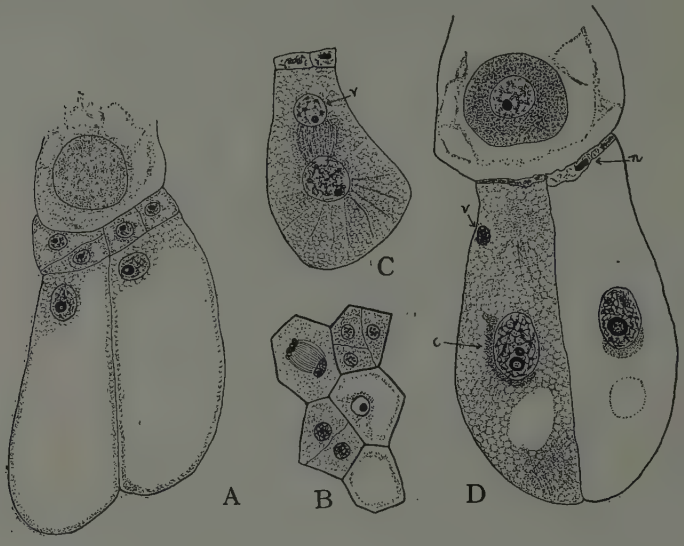

FIG. 3—*Callitris rhomboidea.* **A.** Two young archegonia, with neck cells, in contact with the pollen tube which shows the body-cell cut at one end. × 375. Jan. 5. **B.** Neck cells, in surface view, of three young archegonia. × 375. Dec. 3. **C.** Archegonium with large ventral canal nucleus *v.* × 250. Dec. 21. **D.** Mature archegonia in contact with pollen tube which shows one of the male cells—cytoplasmic condensation round female nucleus *c,* degenerating neck cells *n,* ventral canal nucleus, *v.* × 375. Dec. 21.

Saxton (1910) speaks of the early disappearance of these neck cells. They undoubtedly show early degeneration (Fig. 3 C, D) and sometimes disappearance, but complete disappearance does not seem so common in *C. rhomboidea,* in which species they may persist up to fertilization. The number of the archegonia seems also here greatly in excess of that mentioned by Saxton. More important, however, is the occurrence of a ventral canal nucleus. The mitosis giving rise to it was not seen, but there can be no question of its occurrence. Quite frequently it remained as a large permanent nucleus, almost as large as the egg nucleus. This is shown in Fig. 3 C, in which case traces of the spindle of the recent division were distinctly visible. Rarely, this nucleus must undergo division, as occasional archegonia were seen with two or three nuclei in the upper part quite distinct from the egg nucleus. Ordinarily, it degenerates fairly rapidly and in a normal manner, as shown by the left

archegonium in Fig. 3 D. The division giving rise to it must take place late, as this nucleus is only found in archegonia in which the vacuole of the young stage has been almost obliterated. As the archegonium matures, and the protoplasm begins to fill it from above downwards, a zone of denser protoplasm appears below the nucleus. Such cytoplasmic bodies have been described in many conifers (see Doyle and Saxton, 1933, for a recent list). It is tempting to consider that properly fixed cytoplasm might reveal it as a Golgi body. Here it is uncommonly permanent, and remains as a conspicuous structure closely associated with the egg nucleus up to fertilization. It is not recorded for *Actinostrobus*. It appears in Fig. 3 D, which shows two archegonia ready for fertilization, and in contact with the pollen tube in which one male cell is drawn. The archegonia are very variable in shape, as is natural when so many are tightly grouped. They may be much shorter and wider than those here shown, and are often wide at the base and narrow at the neck; the zone of contact with the pollen tube may be much less definite, due to further disintegration of the neck cells and partial digestion of the pollen tube wall. The egg nucleus is situated in the lower middle of the archegonium, or even deeper. In the case drawn these nuclei are relatively larger than normal. The male and female nuclei normally approximate more closely in size, as Saxton (1910) has described.

Fertilization and proembryo formation.—A good series of stages of these phases in development was available. Owing to the need of war economy many are not reproduced, but photomicrographs are available for examination by any interested. From several preparations showing the actual entry of the male cell, Fig. 4 A is taken from one with the entry well advanced. The male nucleus gets slightly in advance of its own cytoplasm, of which apparently the whole enters the egg. The cytoplasmic condensation near the egg nucleus is still visible. The male cytoplasm, containing numerous starch grains which are evident though still fairly small in Fig. 4 A, initiates the dense starch-containing zone which envelops the fusing male and female nuclei. Saxton (1910) comments on the absence of starch in the archegonium at this stage. Only two archegonia with the nuclei in contact were seen by him, and no other stage from fertilization to late proembryo. He describes, however, as a definite feature plasmic radiations extending from the fusing nuclei (see his Pl. 45, 17). The series of preparations now available show quite clearly that from the earliest stage of fusion there is a starch sheath, probably derived from the male cytoplasm, surrounding the nuclei. This is shown by Fig. 4 B, in which the male and female nuclei are beginning to coalesce, and even more distinctly in Fig. 4 C the late telophase of the first sporophytic division. It is, in fact, quite a characteristic feature at all stages up to the early embryonic phase. The plasmic radiation appearance described by Saxton was met with occasionally, and is easily explained. The archegonial cytoplasm when fixed about the fertilization stage shows this radiation appearance quite commonly (see Figs. 4 A and 3 C). In

the early stages of contact between the male and female nuclei the starch grains brought in from the male cell are still quite small, and can be easily overlooked under common conditions of staining, although they rapidly enlarge later. At

Fig. 4.—*Callitris rhomboidea.* **A.** Late stage of male cell entry, male nucleus in advance of its cytoplasm which contains much fine starch. × 500. Dec. 21. **B.** Male and female nuclei in contact surrounded by sheath with numerous starch grains. × 375. Dec. 12. **C.** Late telophase of first sporophytic mitosis, the starch sheath more definite; a v.c.n. still visible above. × 375. Dec. 18. **D.** Four-celled proembryo. × 375, Dec. 10. **E.** Four-celled proembryo—linear type. × 375. Dec. 3. **F.** Subsequent divisions in two cells of a four-celled proembryo. × 375. Dec. 24. **G.** Symmetrical six-celled proembryo of which the two upper play no further part. × 375. Jan. 5.

the outset they tend to be regularly disposed in the cytoplasm, and, when small and inconspicuously stained, help to emphasize the appearance of plasmic radiations. Saxton (1913) records clearly the presence of a dense starch sheath in *Actinostrobus.*

A second division follows the stage shown in Fig. 4 c, the nuclei dividing simultaneously. Walls are formed at the end of this division resulting in a four-celled proembryo. The appearance of this proembryo is determined solely by the lie and position of the spindles at the second division. A simple regular type is shown in Fig. 4 d. In such a case as this the zone of cytoplasm in the

archegonium above the starch sheath may be hardly encroached on, the proembryo leaving a varying amount unused. This zone rapidly deteriorates, is cut off by the wall formation in the proembryo, is further crushed by the subsequent development of the latter, and so is seldom met with in later stages (but see Fig. 5 C). On the other hand, these four cells may lie with two side-by-side above and two superimposed below; or this may be reversed; and even a linear proembryo may be formed (Fig. 4 E). In this latter case naturally a greater encroachment of the sterile upper zone in the archegonium takes place. This aspect of proembryo formation in conifers in general is of some importance. Statements to the effect that the proembryo fills the archegonium are misleading, however accurate they may be in fact. It is reasonably certain that in no conifer is the whole of the egg used as it is in the early segmentation in bryophytes and pteridophytes. Rather the proembryo is cleaved out, around the fusion nucleus, from a certain amount of the egg cytoplasm, leaving a varying amount of this unused—a condition seen most clearly in the araucarians. The unused cytoplasm may belong to the lower part of the archegonium as well as to the upper. Further, as shown by the araucarians, walls formed in the proembryo are not necessarily connected with the archegonial walls; so, although it has not been demonstrated in the other conifers, yet it is to be expected that the proembryonic cytoplasm is surrounded by walls which are its own, and quite independent of the archegonial walls, although they may be in close contact with these latter. It is difficult to account otherwise for the elongation in the walls of, say, prosuspensor cells, the old wall of the archegonium proper hardly resuming growth in such a case. If further followed out this idea might well be related to the concept recently put forward by Priestley and Scott (1939), that, even in mitosis in ordinary tissue, each protoplast forms its own individual membrane, the membrane appearing on the mitotic spindle not being connected to the existing wall of the parent cell. It can, of course, be readily seen in cellular formation in the prothallus of many conifers, *Saxegothaea*, for example (Looby and Doyle, 1939), that the walls of the outer cells are quite separate from the gynospore membrane.

Further divisions, commonly in two cells, follow in the four-celled proembryo (Fig. 4 F). These may apparently take place in any of the cells resulting in six-celled proembryos, of which a rather regular one a little advanced is drawn in Fig. 4 G. In this case already the upper two cells are much less important than the lower four, with less starch, smaller nuclei, and more vacuolate cytoplasm. These will play no further part, and break down more or less rapidly.

The arrangement here shown is fairly common, but there is no real regularity at the six-celled stage. A few of the arrangements found, and which can be readily derived from typical four-celled stages, are outlined diagrammatically in Fig. 5 A–D. A and B are obvious variants on Fig. 4 G. C is interesting, showing not only a different arrangement of two upper and four lower cells, but also the upper deteriorated zone *a* of the archegonium not

used in the proembryo. A linear row of six, as in D, was occasionally seen. Definitely irregular forms also occurred, of which one rather simple type is drawn in Fig. 5 E. It is doubtful whether many of these develop. Very occasionally a third division in the original four-celled stage produced a seven-

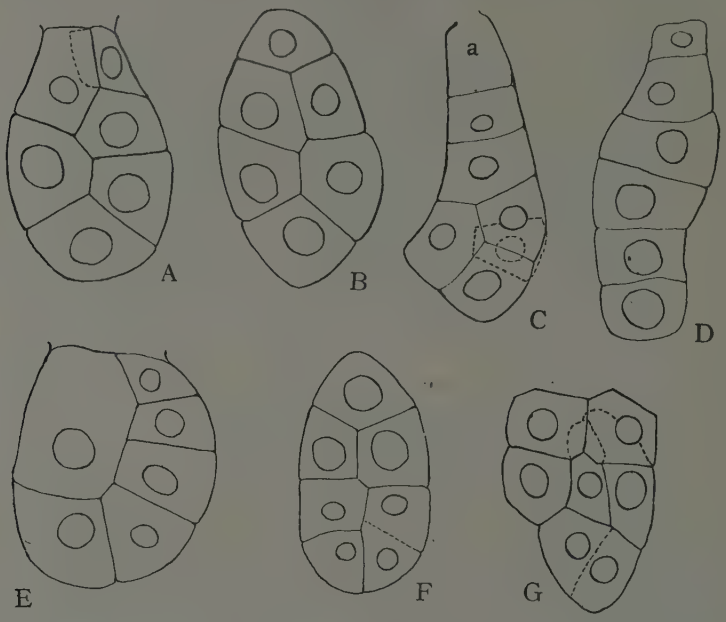

FIG. 5.—*Callitris rhomboidea.* **A–E.** Various forms of six-celled proembryos, **E** definitely irregular, **D** rare. **F.** Regular seven-celled proembryo. **G.** Irregular seven-celled proembryo. All × 375. **A, B, D**—Jan. 5; **C, E,**—Dec. 10; **F, G**—Dec. 16.

celled proembryo as in Fig. 5 F. In this case the lower four cells have probably all come from the lower cell of the early proembryo; their nuclei are enlarging from a recent division. Although only a few seven-celled stages were seen, some of these were definitely irregular, as in Fig. 5 G. A few doubtful five-cell stages were seen.

Saxton's (1910) earlier account of proembryo formation is admittedly incomplete. It is frequently difficult to disentangle the relationship of two developing proembryos which lie together, and if only a little material is available decision may be impossible. However, with the results of this fuller series available, re-examination of some of Saxton's preparations showed that they were in perfect agreement. It is thus clear that the commonest and most typical proembryo is one with six cells, of which the four lowest tend to be more definite than the rest. The formation of walls in the early proembryo at the four-nucleate stage, and the subsequent division of some of these cells to give an upper two of less importance than a lower group, are points of close

resemblance to *Actinostrobus* (Saxton, 1913). The number of cells in the lower group was not definitely stated by Saxton for this genus, but work on the later stages by Miss Baird (1937) seems to show that the number of active lower cells is four, as here found in *Callitris*.

Early embryogeny.—In her paper on the embryogeny in *Actinostrobus* Miss Baird (1937) states that *Callitris* closely resembles it, an account of *Callitris* being promised for another paper. As this paper is not yet available a few points on the early embryogeny in *C. rhomboidea* are given here, leaving the full account and the later embryogeny in the genus to be dealt with by Miss Baird.

Each of the four larger cells in the final proembryo stage divides to form a small outer embryonic cell and a larger inner cell which elongates to form a suspensor. There is no regularity in the direction of growth. A few typical

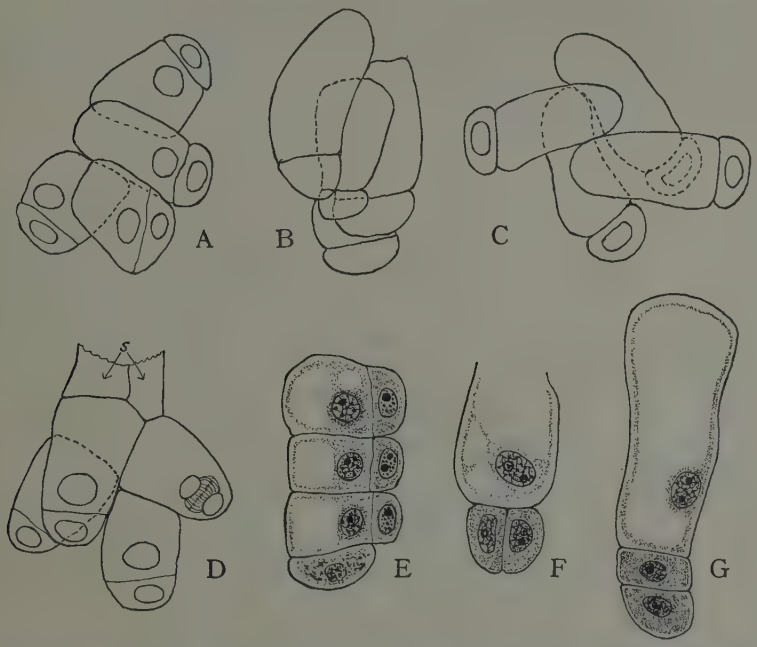

Fig. 6.—*Calltris rhomboidea.* **A–D.** Typical groups of four suspensor cells each with an embryo cell. In **D** the remains, *s*, of two sterile cells still visible. **E.** A linear proembryonic group in three of which the embryo cells were all cut off on the same side. **F** and **G.** Two-celled embryos of which F is typical. All × 375 and Dec. 27 (except D—Dec. 10).

appearances are drawn in Fig. 6 A–D. Commonly the upper sterile cells degenerate rapidly, but in *D* two sterile cells *s* are still shown. This figure also shows that the elongation of the suspensor may overlap the completion of the division cutting off the embryonic cell. Occasional irregularities may

occur at this stage. The proembryo cells may elongate without cutting off any cell, or a series of divisions and elongations may take place with no particular order, and with little distinction between embryo cell and suspensor. These departures from normal were rare. In every one of the numerous examples of normal development the cleavage results in four embryos and suspensors. In Saxton's account of *Actinostrobus,* in which this unique type of early embryogeny was first accurately described, the number of cells so behaving was left doubtful, and the elongation of all the suspensors is described as occurring on the same side of the proembryo. In Miss Baird's later account (1937, Fig. 1) the number is given as regularly four, and the suspensors are described as growing out in any direction; the agreement here with *Callitris* is remarkable. As Miss Baird had plentiful material her account more probably represents the actual position. Fig. 6 E shows, however, a condition once met with. One of a linear group of four active proembryo cells degenerated, but the other three cut off the embryonic cell on the same side; this was the only case resembling at all closely Saxton's Text-fig. 3. Concerning the later stages it need only be said here that the first division is usually, as in Fig. 6 F, vertical, but it may be horizontal as in 6 G.

Conclusions.

Essentially this paper adds those details of development necessary to establish the commonly recognised relationship between *Callitris* and *Actinostrobus.* These details are necessary. In *Sequoia,* for example, two species are usually included, but, in spite of the lateral position of the pollen tube in both, these two species differ so completely in their development as well as in other features that it now seems evident that they must be kept separated into different genera. The development, however, in *Callitris* and *Actinostrobus* is practically identical. Lateral pollen tubes and archegonia, early formation of the outer layer of small cells in the prothallus, equality of the sexual nuclei, wall formation in the proembryo at the four-celled stage followed by a few additional divisions, the peculiar embryogeny—all are important features of similarity; and to these can be added similarity in habit, cones, and geographical distribution. Their relation to the main bulk of the cupressinean conifers raises other questions. *Widdringtonia,* from South Africa, is probably correctly included as a callitrinean type, but there are still too many gaps in its life-history to warrant definite conclusions. The probable development in this form also of a four-celled proembryo and cleavage polyembryony of the *Callitris* type (Baird, 1937) suggests that the relation of these three lies elsewhere than directly with the *Cupressus-Libocedrus* complex. *Fitzroya,* from South America, is, however, another cupressinean conifer with a four-celled proembryo (Doyle and Saxton, 1933) which may possibly show relations with both the cupressinean and callitrinean lines. It has also an outlying supposed relative in *Diselma* endemic in Tasmania. Unfortunately nothing is known of the life-history of *Diselma,* nor has the embryogeny of *Fitzroya* yet been described. When at

least the latter is available it may be possible to reconsider the whole cupressinean group, but, until development in *Diselma* and *Callitropsis* has been worked out, suggestions concerning relationships must probably remain purely tentative.

Acknowledgments are gratefully made to J. W. Besant, Keeper of the Botanic Gardens, Glasnevin, for permission to collect freely from the specimen of *Callitris*, and to W. T. Saxton for the use of his earlier preparations on the genus.

Summary.

1. This account supplements and completes that given by Saxton.

2. Some early stages in the development of the prothallus are described.

(*a*) Division of the nuclei at the vacuolate stage is not simultaneous but occurs in succession from above downwards.

(*b*) The last division is simultaneous and anticlinal to the gynospore membrane, a layer of small outer cells being formed.

(*c*) Alveolar formation based on an ingrowing layer of connected spindles is described, and attention called to this aspect of Sokolowa's work.

3. The pollen tube does not penetrate the prothallus, but may form a deep lateral groove.

4. The body cell and its division, and certain features of the archegonia including the formation of a ventral canal nucleus, are described.

5. Some details of fertilization are figured and described.

6. The proembryo forms walls after the second division giving a four-celled stage, followed by a limited number of divisions. The mature proembryo is typically six-celled.

7. The four lower proembryo cells each divide into an outer small embryonic cell and a large inner suspensor cell which elongates.

8. The close similarity with corresponding stages in *Actinostrobus*, where these are known, is emphasized.

9. The callitrinean genera should probably be related back to some *Fitzroya*-like type rather than to the *Cupressus-Libocedrus* type.

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